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A Summary of Current Program [July 1, 1965]

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and Preliminary Report of Progress

for 7/1/65 to 6/30/66

WESTERN UTILIZATION RESEARCH AND

DEVELOPMENT DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

APR 25 1967  
UNITED STATES DEPARTMENT OF AGRICULTURE

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1965 and June 30, 1966. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Albany, California.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

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## INTRODUCTION

This report summarizes the current program and progress during FY 1966 on research conducted at the Western Utilization Research and Development Division (one of four Utilization Divisions in the Agricultural Research Service). A few of the more significant recent accomplishments are described.

### Research Area Covered by this Report

Utilization research in agriculture deals with the discovery and development of new and improved products from farm crops and the invention or perfection of processing technologies. The scientists, engineers, and technologists who carry on this research devote much of their effort to basic studies of physical and chemical properties of agricultural commodities and products derived from them, in order to provide a firm base of exact knowledge for applied developments.

The farm commodities dealt with in this report are the cereal grains, wheat, rice, and barley; alfalfa and other forage crops; wool and mohair; citrus, apples, other fruits, and tree nuts; potatoes, dry beans and peas and other vegetables; castor and safflower; and poultry and eggs. Some phases of research on certain of these commodities are pursued in Utilization Research Divisions other than the Western Division: The Northeast Division does research on industrial uses of wheat and on milling technology. The Eastern Division has research programs on deciduous fruits and on potatoes and other vegetables. The Southern Division carries out particular lines of research on rice, vegetables, and fruits.

Pharmacological research for all four Utilization Research Divisions is conducted at the Albany laboratory of the Western Division, and is described in the report as it applies to various subject areas. (Progress on commodities assigned to other Divisions should be found in their reports.)

### Aims of Research on These Commodities

The commodities discussed here include a group that provides the nation with more than half of its food, either directly (cereal grains, fruits and vegetables, and poultry meat and eggs) or indirectly through feeding of meat animals (forage crops, wheat, barley). The remaining commodities--the non-food ones--supply us with our most important animal fibers (wool and mohair), or offer opportunities to develop numberless industrial products (castor, safflower, and other oilseeds).

The general aim of utilization research on both of these two broad categories of farm commodities is essentially the same--to provide better and less expensive products for the consuming public and to broaden and extend markets for the commodities, and thereby help to stabilize or increase the demand for them to the betterment of producers and processors.

The fundamental justification for carrying on a publicly supported program of utilization research on food products follows a somewhat different line of reasoning than does the justification for research to extend the utilization of non-food commodities. Research on the processing of farm products for food is justified primarily by its direct benefit to the entire population through improved nutrition and well-being, reduced economic loss from spoilage and waste, and increased opportunity to find profitable markets abroad. Indirectly, too, advances in technology through food processing research bring about major and desirable shifts in the commodity supply and demand picture for the country as a whole, as for example in the economical conversion of abundant feed grains into poultry which is marketable in refrigerated or frozen form throughout the nation and overseas.

Justification for research on non-food products, on the other hand, is based on the avowed public policy of assisting farmers threatened with loss of markets as a result of the swift rise of non-agricultural synthetics, as in the case of wool growers, or on the possibility of developing demand for a presently minor crop to the point where it can be grown profitably on a large scale in order to provide cash crops for diversification and rotation programs and to remove some of the pressure of surplus from other crops--for example, safflower and castor which take land out of cotton production in the western states.

#### Organization of the Division

Research and development along these diverse lines are carried on for the Western Division by a staff headquartered in the Western Regional Research Laboratory, Albany, California. A smaller Department-owned laboratory is operated in Pasadena, California; laboratory space and facilities in Puyallup, Washington, are utilized through a cooperative arrangement with Washington State University, Institute of Agricultural Sciences; and laboratory space and facilities in Honolulu, through a cooperative arrangement with the University of Hawaii.

The Albany research staff is organized into six commodity-oriented Laboratories (Cereals, Field Crops, Fruit, Poultry Products, Vegetables, and Wool and Mohair), two functional Laboratories (Pharmacology, and Engineering and Development), and a Pioneering Laboratory concerned with basic studies of plant enzymes. The staff at Pasadena is organized as the Subtropical Fruit Laboratory. The Western Regional Research Laboratory at Albany also houses the Division Director's staff, the staff required for Administrative support of the Division, and that responsible for Plant Management--that is, operation of the buildings, facilities, and grounds.

Division scientists and engineers not only conduct or supervise research in their own experimental facilities, but also greatly extend the scope and influence of their work by planning and supervising developmental activities carried on by cooperating private firms, processor organizations, or industry groups, and by arranging for research by well-qualified scientists



elsewhere under research contracts and grants. In addition, certain grants of research funds are placed with investigators in foreign countries; the cost of these foreign research efforts on behalf of American agricultural interests is borne by Public Law 480 funds.

### Examples of Recent Accomplishments of the Western Utilization Research and Development Division

WORLD Wheat in World-Wide Tests. WORLD wheat continues to score high in acceptability wherever it is tested. This bran-free, light-colored, whole-kernel wheat is designed to be a low-cost easily used product for marketing in world areas where wheat foods are unknown or traditional forms are less suitable. Extensive follow-up trials in Hong Kong confirm earlier indications of its taste and eye appeal. Nearly a thousand subjects, from 2 to 93 years old, have been eating the product daily for many weeks, and requests are being received for a continuing supply. Tests are also being conducted by Church World Service, the sponsor of the Hong Kong tests, at five locations in India. In small-scale tests by AID mission directors in Korea, Taiwan, the Philippines, Brazil, and Viet Nam, the enthusiastic response prompted requests for larger quantities to amplify the investigation. U.S. and foreign companies alike are exploring commercial production, and at least three U.S. companies are now tooling up to make the product.

Protein-Fortified Rolled Wheat for Immediate Use Overseas. Millions of pounds of whole wheat in rolled or flaked form (similar to rolled oats) are currently going overseas in various food assistance and market development programs. It is very well accepted. A method has now been invented to fortify this product with high-protein flours from wheat, soy, milk, fish, or other sources to make it even more useful for marketing as a low-cost high-quality food protein product. It can easily have a protein content of 20%. The starting grain is wetted, dusted with the high-protein flour, steamed to set the added protein, and rolled in equipment presently used to make ordinary rolled wheat. When fixed in this manner, the added protein does not dust off or separate from the wheat. This fortified rolled wheat is highly palatable, it is quick-cooking, and it can be made easily in large quantity with existing equipment. Acceptability tests underway are most promising--they indicate wide use could be made of this new product.

Food-Protein Concentrates from Wheat Mill Fractions. Department research has shown that high-quality food ingredients can be made from bran and shorts, byproduct fractions produced when wheat is milled into flour. These byproducts have been sold heretofore as animal feeds, but now up to 50% of this material can be recovered as food-grade flours or upgraded feeds. In the new process, the bran and shorts are dried and remilled separately in conventional flour milling equipment. The resulting flours contain 25 to 40% protein and only 2 to 4% fiber. The biological value of the protein is high because it contains more than 4% of the scarce amino acid lysine. The flour milling industry has used these research results to develop a high-protein, general-purpose flour and a product that can be made into a nutritious gruel or beverage to supplement protein-deficient diets.

Mill byproducts available are sufficient to allow recovery of nearly two million tons per year of these high-protein concentrates.

WURLAN II, An Improved Finishing Treatment for Wool. A new textile-finishing process, WURLAN II, extends considerably the range of polymer finishes that can be applied to wool. The process involves technology similar to that employed in the original WURLAN process. In WURLAN I, monomers are used to form polymers on wool; in WURLAN II, preformed polymers are used. The new process produces desirably soft, durably shrink-resistant wool fabrics, and the polymers used are low-cost and commercially available. Since many types of resins can be used, WURLAN II is particularly useful for developing multipurpose effects, such as soil resistance combined with shrink resistance.

New Soil-Resistant Finishes for Wool. Treatment with fluoropolymers provides the most effective soil-resistant finish for textiles, but these polymers have been exceedingly expensive. Now an entirely new synthesis has been discovered which gives promise of providing superior soil-resistant finishes at less than half of the cost of previously available treatments. From hexafluoroacetone, several new families of polymers have been made, including polyfluoroacrylates and polyfluoroethers. When these polymers are dissolved in the proper solvents, they can be applied easily to fabrics or finished garments to provide water- and soil-resistance that lasts through repeated laundering, drycleaning and wear. Some of the new fluoropolymers also make wool shrink-resistant; thus the way is open to new multipurpose finishes. The fluoropolymers can be used in combination with the WURLAN I treatment, which is now being widely used commercially to make wool machine-washable.

OSMOVAC Fruits--Superior Dehydrated Products. Department scientists have developed an entirely new concept for drying fruit. By combining the techniques of osmotic dehydration and vacuum drying, they have produced dried fruits of superior flavor and color. The name OSMOVAC is applied to the process. Fresh fruit, whole or sliced, is covered with dry sugar or a heavy sugar syrup and allowed to stand for a few hours, then it is drained and further dried under vacuum. The sugar removed about 70% of the water in the fruit. It also protects the color and flavor throughout the process so that no sulfite, as required in conventional dehydration, need be used. The process has been applied to whole and sliced strawberries; sliced bananas; cut apricots; sliced peaches, pears, and papayas; blueberries; and pitted cherries. The dried products are bright and colorful as well as delicious. They are attractive as confections and for use in breakfast cereals. Three major cereal manufacturers are evaluating OSMOVAC dried fruits for possible inclusion in dry breakfast cereals.

Instant Applesauce Flakes. A new convenience food, instant applesauce flakes, is now in commercial production. One company with orders exceeding its production capacity is preparing to double its output next season. Other companies are investigating installation of the modified drum drier developed by Department engineers for drying applesauce and other fruit purees. The modification consists of adding a chilled air blast to facilitate removal of flakes from the hot drum surface. Production costs are low because drum

drying is one of the simplest and least expensive of drying methods. The applesauce flakes can be used in cake mixes, baked desserts and pastries, candy and apple butter, and as fruit in breakfast cereals. The flakes reconstitute quickly in cold water to make an applesauce of excellent flavor, color and texture. They meet a long-standing need as a military ration item, and, because of their high quality, they were used recently in manned space flights.

Sweeteners from Citrus Fruits. Citrus fruits contain a wide array of complex compounds known as flavonoids, some of which cause bitterness in the juices, particularly in grapefruit juice. While Department scientists were studying the chemistry of these bitter compounds to learn how to process citrus to eliminate this bitterness, they made the important discovery that suitable chemical treatments of the two most abundant bitter flavonoids, naringin and neohesperidin, could reduce bitterness, eliminate it entirely, or even produce sweet-tasting products. Naringin can be converted into a substance as sweet as saccharin, and neohesperidin into one that is 20 times sweeter. The sweetness of both products is pleasant and long-lasting, without the metallic after-taste of saccharin. Over two dozen chemical, pharmaceutical, and food companies have obtained samples and are evaluating these compounds for use as non-caloric sweeteners in such products as dietetic soft drinks and medicines. The method for preparing the sweeteners has been patented by the Department. Work continues on developing a feasible chemical debittering process for citrus juices.

Processed Hawaiian Fruits for Mainland Markets. During the past several years in Hawaii, an increasing number of acres have been planted to tropical and subtropical fruits in order to diversify an agriculture that has been limited largely to sugar cane and pineapple. Papaya, guava, and passion fruit are now grown commercially. Department scientists in cooperation with the Hawaii Agricultural Experiment Station are developing high-quality processed products from these crops, which traditionally are consumed as fresh fruit.

An improved method for separating the skins and seeds from papaya yields a puree which has a superior flavor because it does not contain any bitter taste from the peel. This puree is a convenient raw material for jam and jelly manufacture. Enzymes can be used to make fruit purees more suitable for concentration and juice making, a finding that is expanding the use of tropical fruits in mixed fruit juices. Recent research shows that bananas, guavas, mangoes, and papayas can be dehydrated with great success. Samples now being evaluated by Hawaiian processors have been described by taste panels as having excellent flavor. These new stable forms of tropical fruits will open markets all over the world for these delicious commodities--to the benefit of consumers and manufacturers--and provide a new basis for Hawaiian prosperity.

Control of Consistency of Tomato Products. Research by Department scientists has led to the successful application of fundamental information about enzyme behavior to the practical problem of controlling the consistency of tomato products. Approximately three-fourths of the tomato crop is processed



into juice, ketchup, sauce, paste, or some other pureed product for which consistency is an important quality factor. By adjusting the acidity of raw tomatoes during crushing, it is possible to control the activity of the two enzyme systems which govern consistency, so that tomato products of unusual properties can be produced. Changing the level of acidity allows the processor to make high consistency juice, low consistency juice, firm tomato juice gels, or products like catsup or sauce with intermediate consistencies. The Department has filed two patent applications to cover these processes. In addition these findings provided the basis for a new assay method for raw tomato "consistency potential." This method is useful to industry in selecting new tomato varieties for processing, matching raw material characteristics to end-product requirements, producing more uniform processed products, and studying the effects of other processing variables on end-product consistency. Extensive commercial tests of the assay method are planned for the 1966 tomato harvest.

The WURLING Evaporator--A New Vacuum Concentrator for Tomato Paste. Thick fluids, like tomato paste and fruit preserves, are difficult to concentrate because they foul the evaporators with burned or stuck material. Through basic research on fouling, Department engineers have designed the WURLING evaporator for concentrating thick and heat-sensitive fluids. A rapidly whirling coil is completely submerged in the liquid under vacuum. Both submersion of the coil and its movement in the fluid help to reduce fouling. By operating under vacuum, low-temperature steam can be used in the coil, thus permitting accurate temperature control and preventing the development of hot spots. Fouling is minimized and quality of the product is preserved. Tomato paste with 50% solids and having excellent color and flavor is now being produced by one processor. He is using a WURLING evaporator that can produce 4.5 tons of product per hour. Another processor is making high-quality fruit preserves with a large WURLING evaporator. Cost savings are considerable because the WURLING evaporator doubles output at less than half the cost of conventional thin-film evaporation. One equipment manufacturer makes the WURLING evaporator now, and others may be in production soon.

Reduction of Heat Resistance of Bacterial Spores. In canning non-acid foods, severe heat treatments have had to be used to destroy bacterial spores, which are remarkably heat resistant. If a way could be found to reduce the heating time, canned vegetables would have much better color, flavor and nutritive value. In basic research aimed at understanding how spores resist destruction by heat, Department scientists discovered a previously unrecognized ion-exchange property of spores that determines their heat resistance. The spores can be sensitized to heat by an acid treatment, and this sensitivity persists when the product is returned to its original non-acid state. Because spores behave in this way, less severe heat treatments might be used for sterilization. A public service patent on this method of reducing the severity of heat sterilization treatments has been applied for. Efforts are now being made to apply this invention to specific products. If these efforts succeed, we will have better canned peas, sweet corn, green beans, and squash, as well as new canned products such as cauliflower and broccoli, which are too tender to withstand current commercial sterilization.



Quick-Cooking Dry Beans. A simple new process for making convenient quick-cooking dry beans promises to stimulate a much needed expansion in the market for this high-protein food. Research on the product was partially supported by the California Lima Bean Advisory Board. The new product, to be marketed in a dry form, can be prepared for table use in about 30 minutes by cooking in boiling water. The cooked beans have excellent appearance and flavor. Several food manufacturers are interested in using the process, which is applicable to other varieties of dry beans as well as Limas.

High-Protein Food from Safflower Meal. Safflower meal is a rich source of vegetable protein, but cannot be used directly as a food. The fiber content is three times the acceptable level, and the meal contains a bitter flavor which is not destroyed by cooking. The Department has now developed processes that overcome both problems. Safflower seeds are partially decorticated by screening and air classification, the oil is removed by pressing and solvent extraction, then more fiber is removed by dry milling of the oil-free meal. The resulting product is about 60% protein and 3 to 5% fiber. At this stage it is immediately useful as a superior animal feed ingredient. If the bitter flavor is then removed by extracting with alcohol or acetone, the protein content is increased to about 75% and the flavor becomes desirably bland. Preliminary results indicate that this high-protein safflower meal can be incorporated in bread or in meat-substitute dishes to make nutritious, palatable foods. The critical shortage of protein foods in many parts of the world underscores the importance of these findings.

Superior Cooking Oil from New Variety of Safflower. Department scientists, working in cooperation with plant breeders at the California Agricultural Experiment Station, have shown that a new variety of safflower with high content of oleic acid provides an oil that is comparable in quality with the best hydrogenated oils presently used in deep fat frying. High-oleic safflower oil is stable to air oxidation without being hydrogenated and remains fluid at refrigerator temperatures. Thus processing and handling costs are low. Many large commercial suppliers and users of edible oil in this country have requested samples of the new oil and detailed information on this work. The potential use is enormous--one of these users alone consumes 100 million pounds of frying oil each year.

Economical Method for Producing Industrial Chemical Opens New Markets for Castor. Highly efficient methods have been developed for converting low-cost hydrogenated castor oil esters to ketostearate esters, which have unique physical and chemical properties that should make them industrially valuable. Several companies are evaluating the ketostearates for such uses as permanent-type automotive lubricants and mold-release compounds. The latter are mixed with polyethylene so that molded products such as phonograph records are released quickly and easily from the mold without sticking. Inexpensive catalysts rapidly and completely convert castor esters to ketostearates. Processing costs are only a few cents a pound. One industry spokesman foresees an immediate yearly market for several million pounds of ketostearates in lubricant applications. Another large producer has embarked on an extensive exploratory research program to develop uses for ketostearates, now that they can be prepared by this low-cost method.

Measurement of Cohesiveness in Poultry Meat. A method has been developed for the mechanical measurement of the cohesive force holding fibers together in poultry meat. It provides, for the first time, an objective measurement of a texture defect found in canned, freeze-dried, and irradiated meats and associated with an undesirable crumbly, cottony feel in the mouth. Adequate cohesiveness is recognized as essential for good texture in meats. The method employs standard tensile-testing equipment and an adhesive compound that forms a bond between metal and poultry meat stronger than the cohesive force within the meat. Cohesiveness measured by this method correlates well with the degree of degradation of the connective-tissue protein, collagen. This method for measuring cohesiveness added to the shear force method of measuring tenderness provides two valuable tools for research to improve texture of processed poultry products.

As a step toward implementation of the recommendations for a National Program of Research for Agriculture made jointly by the Association of State Universities and Land Grant Colleges and the USDA, a section has been added to each of the Areas in this report. It comprises a list of the related publications of the State Agricultural Experiment Stations in addition to those heretofore reported covering the results of USDA and cooperative research. In future years, it is anticipated that information will be available to permit reporting of achievements resulting from State research in a format comparable to the present reporting of the USDA and cooperative research.

#### Examples of Recent Accomplishments of the State Agricultural Experiment Stations

New high-solids tomato product developed. A new high-solids tomato product that resists spoilage by yeasts, molds and bacteria and has a greatly extended shelf life has been developed by California researchers. The new product was developed for use by remanufacturers in such items as soups, relish, sauces, Italian dinners and a variety of other products in which tomatoes are an essential ingredient. It can be easily reconstituted to the desired solids content and is economical to transport. In the procedure, fresh canning tomatoes are washed, crushed, and the juice extracted by conventional means. The extracted juice is then concentrated under vacuum to about 50 percent solids, at a temperature low enough to minimize damaging heat effects on the products. From the vacuum concentrates, one method employs a valve which extrudes the concentrated paste through a stream of hot air onto a tray or belt. This extruded product is spaghetti-like in appearance and when the desired degree of dehydration is attained is dry enough to be ground into a powder.

A continuous-crush press developed for the grape industry. California station scientists have developed a new continuous-flow fruit press for wineries. Flexibility of layout, light weight and low power requirements allow the press to be built in a large number of configurations and sizes. Average yields of liquid from grapes increased 9 percent above the conventional basket-pressing



method. This machine has been patented and will be produced by commercial manufacturers. Further evaluations of the press are planned with grapes and a variety of other products.

Wine colored by steam passes test. Port wine colored by the steam-heat process developed by California scientists proved to be as good as or better than conventional port. Results indicate that the steam treatment may be useful in all red wine production. The mechanized, high-speed pressure cooker was designed to extract the red skin color from fresh grapes in a hurry and without lowering the quality. Workers found that grapes steam-treated only 15 seconds in the new cooker, easily matched the fermentation-colored wines. Meanwhile, the food industry is showing interest in the mechanical cooker, which looks promising for precooking (blanching) frozen foods.

Onion dehydration studied. Factors required to produce a high-quality dehydrated onion are under investigation at the New York (Geneva) station. High solids content, high pungency, uniform color, large size and globe shape, ease of peeling and storability were factors needed to produce good dehydrated onion products. New York varieties were found to comply with existing color standards suggested by the American Dehydrated Onion and Garlic Association.

Accelerated water-uptake in dry pea beans. An investigation of the water-uptake in dry pea beans (*Phaseolus vulgaris*) was undertaken by Indiana scientists to explore the possibilities of shortening both the soak process and the thermal process as practiced in commercial canning. Rate of water-uptake increased when adsorbed or trapped gases were released from the surfaces of the beans by steam pressure, vacuum or sonic treatment. Water-uptake rate depended on the thickness of the layer of beans being treated. Certain combinations of polyphosphates and sodium chloride resulted in greater water-uptake than did corresponding concentrations of polyphosphates alone. Lack of sufficient bean tenderness continues to be the main problem in shortening the thermal process. However, beans soaked with both polyphosphates and salt and in less than half the conventional processing time were found to be more tender than conventionally-processed beans.

Fate of bacteria in chicken meat during freeze-dehydration, rehydration and storage. Scientists at the Georgia station have studied the fate of bacteria in chicken meat during freeze-dehydration, rehydration and storage. Survival of the natural flora was determined after the meat was freeze-dehydrated and rehydrated at room temperature for thirty minutes, at 50, 85 and 100° C. for ten minutes. Total counts of bacteria in the rehydrated samples of meat were determined during storage of the meat at 4, 22, and 37° C. until spoilage odor was detectable. Meat samples were inoculated with *Staphylococcus aureus*, then dried, rehydrated and stored at the same room temperature. Numbers of surviving organisms in the inoculated samples were determined with use of both selective and non-selective media. The study indicates that freeze-dehydrated meat should be produced with adequate microbiological control and

that such meat should be rehydrated in very hot water since survival of vegetative cells of potential food poisoning or food infection organisms is a distinct possibility in freeze-dehydrated meat.

Detection of Salmonella in eggs and egg products with fluorescent antibody.

Salmonellosis is one of the important food-borne infections in the world today. Iowa station scientists studied the usefulness of the fluorescent antibody technique for rapid Salmonella detection in dried egg products. Organisms of the genus Salmonella are detected in eggs and egg products within 24 hours in the presence of Pseudomonadaceae and other Enterobacteriaceae by combining selective cultural methods with fluorescent antibody techniques. These techniques are specific for Salmonella when H antibodies are used. Absorption techniques are necessary before the O antibodies give specific reactions for Salmonella. No cross-reactions appear when H antiserum is used. Absorption and interference techniques indicate the list is specific for Salmonella. Work was supported in part by grants from the U.S. Public Health Service and the USDA.

## AREA NO. 1. WHEAT UTILIZATION - FOOD

Problem. The dominant feature of the wheat economy in the United States continues to be a production capacity that can outpace consumption, including the substantially expanded foreign markets of recent years. Increased exports of wheat from the United States in the last three years have brought our wheat carryover to a level that provides less than a prudent reserve. However, the capacity to produce wheat in this country is still restricted.

We view this North American surplus capacity as an unparalleled opportunity. Wheat in excess of domestic needs can be used to buy time in the overpopulated areas of the world until a self-sufficient agriculture can be developed there. Export donations and concessional sales of 600-800 million bushels per year are providing food where it is most needed in the world. This distribution of wheat serves immediate Defense and State Department missions and also stimulates a long-range market development for United States agriculture. New wheat foods specifically adapted to conditions of use in every region of the world would help materially to popularize this valuable food grain in areas where it is now virtually unknown, and development of simplified methods to process the products at the point of use would speed their adoption.

We also need to increase the commercial exports (currently less than 200 million bushels annually) that contribute favorably to our international trade balance. New processes to elicit maximum quality performance of wheats and flours in products produced in Europe and Japan would help significantly to promote trade in these dollar markets. Sustained further gains in wheat markets are necessary to ease governmental restrictions on production more than they have already been eased, and especially to strengthen export trade balances. Increased world supplies of wheat and restrictive political decisions in the European Economic Community have contributed to seriously reduced commercial exports in some years. Everything possible must be done to increase total wheat markets, but especially those in which payments are made in dollars.

Consumers of wheat foods in this country have benefited greatly by introduction of a wide variety of new and improved products. Well balanced diets, reasonable food costs, and improved convenience result from such developments and are suitable objectives of research. Research programs along these lines would sustain and increase markets for wheat.

An essential foundation for a successful product and process development program is basic research on the composition of all classes of wheat and the fundamental properties of their constituents. This kind of information provides the foundation for improved and new products and processes.



## USDA AND COOPERATIVE PROGRAM

Research on utilization of wheat for food seeks to solve the most urgent problems hindering the development of markets for the full productive capacity of U.S. agriculture. The emphasis is on (1) expansion of overseas dollar markets for U.S. wheats; (2) development of new wheat food products for long-term market development in food-short nations abroad; (3) raising the domestic consumption of wheat foods by increased variety, quality, and convenience; and (4) finding means to upgrade wheat millfeeds to recover fractions of nutritious food quality. Basic research on the fundamental chemical and physical properties of wheat and barley constituents and on the functional properties of wheat flour constituents supports the product development and problem-solving segments of the program.

Research is conducted by the Western Utilization Research and Development Division at Albany, California; under contracts and grants at Pullman, Washington; Chicago, Illinois; Manhattan, Kansas; Madison, Wisconsin; St. Paul, Minnesota; Menlo Park, California; and Corvallis, Oregon; and under P.L. 480 grants in England, France, Poland, Italy, Australia, Switzerland, Israel, India, Japan, and Belgium.

The Federal program of research in this area totals 27.0 scientist man-years and 8 contracts and grants providing research at a rate of approximately 7.1 scientist man-years per year. Of this number, 7.5 are assigned to investigations on chemical composition and physical properties; 4.7 on flavor; 5.6 on color, texture and other quality characteristics; 0.2 on microbiology and toxicology; and 9.0 on technology - process and product development. In addition, the Division sponsors 13 research grants under Public Law 480.

### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 22 scientist man-years is devoted to this area of research.

### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

#### A. Chemical Composition and Physical Properties

1. Proteins of Wheat. We study wheat proteins and components that associate with them because the unique character of wheat protein is essential in making a strong, smooth, resilient dough and baking a bold attractive loaf of bread. The spread of cookies, the formation of cake crumb, and the texture of crackers also depend considerably on the amount and character of wheat protein, although each demands differences in these factors from those ideal for bread making.

In former days when baking was a hand operation, the baker adjusted his formula and dough manipulation to correct for differences in the quality of the flour he used. If a dough was sticky, the baker would just add a little more flour; if it was a little tough he would add more liquid or shortening

or increase the mixing time. The need then to control baking quality of flour was not as great as it is now. In large-scale mechanized bakeries with mechanical continuous dough mixing, the skilled hands of a baker are no longer available. Uniformity of raw material is the only way to provide efficiency in the baking operation. This is the reason we undertake basic studies of the constituents of wheat and flour and the relationship of constituents to the baking quality.

Of the individual constituents of wheat flour, the proteins have been, and still are, receiving the greatest attention from cereal chemists. Aside from the nutritional importance of flour protein, it is known that the physical and structural characteristics of doughs, batters and all baked products made therefrom are, in large measure, determined by the nature of flour proteins. It is not yet known, however, how the proteins exert their effects.

We study wheat proteins by investigating differences in their composition, physical properties, and chemical reactivities. Individual proteins are separated by differences in solubility, size, and electrical charge by means of solvent differentiation, column chromatography, and electrophoresis. They are characterized by composition, specific immune reactions (antigenicity), and specific enzymic properties.

We have compared soluble and gliadin proteins from hard red spring and durum flours, flours which differ markedly in baking performance and in protein content. From these dissimilar flours, all antigenic proteins of similar migration rate under electrophoresis showed immunochemical cross-reaction, whereas proteins of dissimilar migration rates did not. From this we conclude that individual antigenic proteins of similar migration rate in the two wheat types must be similar in composition and structure. All this means is that the soluble and gliadin proteins are the same in all wheats. Differences in baking performance must, then (1) be related to amounts of protein components rather than type of protein, (2) be influenced strongly by non-protein components, or (3) reside completely in the insoluble glutenin protein fraction.

More accurate quantitative estimates of the distribution of various proteins amongst flours now appear to be a major goal for research. It is possible, however, that non-antigenic portions of the protein molecules may cause differences in properties. Of immediate need for attention are further studies of enzymic activities of separated proteins.

Dr. Grabar, working under a P.L. 480 grant at the Pasteur Institute in Paris, is using the immunochemical methods of analysis that he pioneered to compare proteins in various cereals. He has shown that several of the proteins in barley, rye, and wheat are identical in their immunochemical reactions. Proteins in maize and oats were formerly thought to be in the same group as the barley, rye and wheat proteins, but they are only partially similar. He has shown that one group of proteins in malt is derived from a similar

group existing in the barley prior to malting, even though the changes that occur during malting tend to obscure this fact when proteins are characterized by other methods. He has shown that the parts of certain protein molecules that are responsible for antigenic specificity are not the same parts of the molecule that are responsible for specificity of enzymic activity.

At the University of Poznan in Poland, a P.L. 480 research grant project on the dependence of wheat enzyme activity on sulfhydryl groups has been concluded. The relationship between sulfhydryl groups and enzyme activity is well known, but details of this relationship in cereal enzymes had not been previously reported. In this study, protein-splitting enzymes of wheat flour were isolated and characterized, particularly in regard to their sulfhydryl and disulfide contents. Observations were made on the stability of sulfhydryl groups in whole and ground wheats and malted wheats during storage, on changes in sulfhydryl content during soaking prior to germination, and on comparative effectiveness of various sulfhydryl blocking reagents added to flour or ground whole grain suspensions. A purified enzyme, protease A, was studied. Its sulfhydryl groups were detectable only in the presence of ammonia, a finding that may lead to a chemical characterization of just how the sulfhydryl groups may be masked in proteins, where we know their presence is essential to enzymic activity.

We are studying a protein fraction from wheat flour that makes up about 25% of the gel fraction that remains undissolved when a flour or a dry dough is exhaustively extracted with dilute acetic acid under mild conditions. The protein in the gel fraction represents about 15% of the total protein of the starting flour. So far little has been learned about this fraction, which appears to be part of the glutenin protein, because it is difficult to handle and is insoluble. We have learned that the fraction becomes partly and progressively soluble when a dough is mixed severely, and that its quantity varies among flours of different baking performance. We have found solvents that separate most of the carbohydrate from the gel fraction protein, hence our earlier supposition that this protein was combined in some way with carbohydrate was not sustained. We are now able to prepare a protein fraction that has only a small amount of the carbohydrate xylose included. By column chromatography and gel electrophoresis on agarose, preliminary separations of individual gel fraction proteins are being made. When enough material has been separated, chemical analysis and measurement of physical properties of the separated components will be made.

A related study of the soluble pentosans associated with proteins of wheat flour is being conducted at the Swiss Federal Institute of Technology at Zurich under a P.L. 480 grant. The chemical linkages between pentosans, proteins, and polyphenolic constituents are being characterized. Two approaches are being used. In one, a highly active and specific xylan-splitting enzyme that has been obtained and purified will be used to degrade the pentosan chain that is assumed to be the backbone of the wheat glycoprotein of interest. In the second approach, model compounds are being



prepared that are expected to be similar to the natural glycoprotein. When starch and caffeic acid were used, a high degree of esterification was achieved (i.e., every third glucose unit of the starch was esterified with caffeic acid). The high degree of esterification resulted in a loss of water solubility, so the capability of this model compound to form a gel could not be tested. Similar compounds with lower degrees of esterification will be prepared, and also model compounds in which polyphenols will be esterified with galactomanan and arabino xylan (a natural constituent of flour). Ferulic acid, also a natural constituent of flour, will be used in place of caffeic acid in future research.

The Research Association of British Flour Millers at St. Albans, England, is supported by a P.L. 480 grant to study means of solubilizing gluten proteins so their component parts may be better understood. They have shown that a cupric-bisulfite reagent can be used to solubilize gluten, and they are now studying the effects of this treatment on the proteins. Although they managed to separate the sulfitolized flour proteins, the separated components had a strong tendency to reaggregate. Considerable effort has been spent on finding reagents that will reduce the tendency, which interferes with characterization of separated components. Measurements have been made of the disulfide and thiol groups that are accessible for reaction with sulfite on the separated components. There was a greater accessibility of disulfide groups in a strong-bread making flour than in a weak flour.

P.L. 480 research funds are supporting another attempt to study insoluble gluten by application of ultrasonic vibrations to suspensions of the protein. The work is being conducted at the National Institute of Agronomic Research in Paris, France. An interesting side issue has developed out of this research: they have found that ultrasonic treatment of flour suspensions results in dough formation. One wonders whether the properties of dough formed by ultrasonic vibrations would differ from those in which mechanical mixing is used, and whether some commercial application of ultrasonic treatment would be valuable in continuous dough mixing. In this research, the somewhat different procedure for separating soluble from insoluble proteins produces results that agree with results obtained by other methods, and thus the foundation for further development of this unique method of separation is sound.

A basic investigation of the nitrogenous components of wheat germ is being conducted at the University of Bologna, Italy, supported by P.L. 480 funds. A thorough study of the compositional features of proteins and lipids in wheat germ is underway. Prolamines and glutelins have been found in commercial wheat germ; their presence could be the result of a failure to separate completely the germ tissue from the endosperm during milling. However, in gel electrophoresis of the germ protein preparation, a single albumin component has been reported. Since the endosperm has a number of albumin components, verification of this albumin separation may indicate a suitable method for isolation of germ from endosperm and also may reveal a notable contrast between the proteins from the two portions of the wheat kernel.

2. Maturation of Wheat Flour and Related Chemical Research. Some types of flour, particularly those made from hard red winter wheat, require the addition of oxidative improvers or lengthy storage periods for natural maturation. Oxidative improvers have been found that are widely regarded as safe. Even so, regulations in some continental European countries prohibit the treatment of food materials with almost any chemical agent, so the flour-maturing oxidizers common in America and the United Kingdom are not allowed. Such regulations hinder the development of commercial markets for our most abundant wheats. We are engaged in research aimed at achieving a better understanding of the maturation process, with the long-range objective of finding acceptable alternative methods for maturing wheat flour.

In contract research at Kansas State University, a number of varieties of hard red winter and hard red spring wheats are being studied for chemical composition and responsiveness to potassium bromate and other maturing agents to improve baking strength. Eight hard red winter and five hard red spring varieties from both 1963 and 1964 crops have been tested. The 1963 hard red winter wheats required more bromate, ascorbic acid, and azodicarbonamide than did the spring varieties. However, with potassium persulfate and acetone peroxides, responses were essentially equal. Such results suggest that the improving effects may involve more than one mechanism. Data indicate that hard red spring wheats contain more total lipids than do the hard red winter wheats, with the principal difference being in nonpolar lipids.

We have demonstrated that amino acids form tightly bound complexes with lipid components and metallic ions. This finding supports the hypothesis that metallic ions participate in lipid binding in bread doughs. We have observed that volume changes accompany the formation of such complexes. The volume change should be a useful measurement for estimating the amount of these metallic ions in solution and the stability of the complex.

Oxidative enzymes appear to be related to the mechanism of flour aging. We are supporting contract research at the University of Wisconsin to detect the presence of various oxidation-reduction and other enzyme systems. A wide variety of oxidizing and reducing enzymes have already been shown to be present in whole wheat, in flour, and in several mill fractions. Lipoxidase is the one enzyme that has shown the greatest amount of activity. This enzyme is responsible for oxidizing lipids which are of importance in their chemical relationships with proteins and their effects on maturation of flours and mixing quality of doughs. When the activity of lipoxidase is high, we find that mixing tolerance is low, with the result that the dough breaks down quickly. If these preliminary findings are found to be general, lipoxidase measurement could become an important procedure for describing flours in the specifications from which they are purchased. Quantitative analysis of nine dehydrogenase systems have been run on whole wheat and mill fractions of two hard red spring and two hard red winter wheat varieties. Similar assays were carried out on oxidative enzymes of these samples, including peroxidase, catalase, polyphenol oxidase, and cytochrome oxidase.

Peroxidase and catalase activities were particularly high in all wheats. The spring wheats, however, contained nearly twice as much oxidative activity as did the winter wheats. Data on the dehydrogenases showed a similar trend, so that the two spring wheats tested had a higher overall enzyme activity than did the two winter wheats.

The lipid compositions of whole wheat, ground wheat, flour, bran, and other milling fractions are under investigation at the French School of Milling in Paris, supported by a P.L. 480 grant. The fatty acid composition of wheat lipids in a number of market classes of United States wheats is being analyzed. Further, the effects of wheat lipid components on dough-mixing characteristics are under investigation. To date much of the work done under this grant has been concerned with improving analytical methods and obtaining compositional data on the free fatty acids of the various wheats and their mill fractions. Preliminary correlations of Alveograph resistance measurements with composition of flour indicate that if the total triglyceride lipids are removed from the flour dough, resistance increases considerably. If the lipids are added back to the flour, resistance properties are nearly recovered. The free fatty acids and the acetone-soluble part of the lipids do not affect the resistance, but the unsaponifiable lipids and the acetone-insoluble matter increase it. If just the acetone-soluble and the insoluble parts are added back to defatted flour, higher Alveograph resistance is observed than if all the extracted lipid material is added back. If the dough is allowed a 20-minute rest period between mixing and testing with the Alveograph, results are not all the same as those observed without resting.

Under a P.L. 480 grant the National Institute for Agronomic Research in Paris, France, is conducting a study of the lipase activity of wheat in relation to water-vapor tension, partial pressure of oxygen, and temperature. Lipase activity by some measurements increases appreciably during germination. The advance of knowledge on lipase activity in wheat and on the evolution of lipase action in germinating wheat is hampered by the low intensity of lipasic activity and by a lack of specificity in the analytical methods that have been developed. Therefore, work on this project has been largely centered on studying chemical methods and adapting them so as to obtain greater sensitivity and, therefore, more valuable data. New microanalytical methods using thin-layer chromatography and gas chromatography are being developed. Free fatty acids released from wheat substrates by lipase activity were found to be in the same proportions as those existing in olive oil glycerides. The maximum lipase activity in germinating wheat is located in coleoptile tissues. Calcium ions are essential to the lipase activity. Proteins were extracted from wheat seedling coleoptiles but only a relatively low yield (10-20%) of the lipase activity was preserved in the protein isolation. Perhaps protein-splitting enzymes were active during the extraction procedure; they could have split the lipase protein and rendered it inactive.

The relationship of wheat flavans to various enzyme systems present in wheat flour is being studied under a P.L. 480 grant at the Agricultural Higher School in Poznan, Poland. Accurate methods for extracting and measuring the



flavans of wheat have been developed and an instrument for flavan determination has been designed and constructed. Equipment available previously for fluorometric analysis was not applicable to tightly bound flavan. The new instrument is capable of precise determination of minute flavan fluorescence in an abundance of interfering substances. The total flavan contents and the free riboflavin ratios have been estimated for two spring and four winter wheats and the flour from them. With the new or improved analytical techniques, studies will now be conducted on the influence of flavans on some enzymes of technological importance in flours, and on the effects that storage of wheat and flour has on flavan components.

Another project supported by P.L. 480 grant funds at the National Institute of Agronomic Research in France concerns the behavior of wheat proteins when they are associated with phosphorus-containing compounds. It has become apparent recently that phosphorus-containing fats (phospholipids) can interact with proteins to form new compounds having properties greatly different than those of the uncombined proteins. Phosphorus-containing phytic acid often forms complexes with proteins. With animal proteins, such complexes are almost completely insoluble, but phytic acid complexes with wheat proteins are acid-soluble. This finding may shed light on the properties of wheat gluten, which is insoluble. Studies of the reactions of gluten with phospholipids and phytic acid may be helpful in gaining a better understanding of how gluten acts in forming doughs, batters, and pasta.

## B. Flavor

1. Chemistry of Bread Flavor. We are investigating the chemistry associated with bread flavor to find means for enhancing and stabilizing flavor so that bread and other baked products will be more satisfying and desirable for consumers. By smelling the effluent from a gas chromatographic column during analysis of a stable bread-aroma concentrate, we have detected three fractions (only one of which was in sufficient quantity to create a peak on the chromatogram) that have cracker-like aromas similar to that reported as resulting from the reaction of proline and dihydroxyacetone. When we reacted proline with glycerol a similar aroma was produced. Both of these reaction mixtures and the naturally occurring aroma components all separate into three fractions having identical gas chromatographic retention times. A tentative chemical identification has been designated for all three fractions. Baking experiments were conducted with doughs to which the reaction mixture of proline and glycerol was added. The addition improved flavor and aroma of the bread.

Studies of volatile constituents in cell-free prefermented broths revealed eight different alpha-keto acids that almost certainly contribute to the overall aroma produced in commercial bread baking. Three of the acids in the preferment increased with time of incubation. The other five occurred in very small amounts so that quantitative determinations were not reliable.

2. Instability of Wheat Product Flavors. Research on food supply for fallout shelters is funded by transfer from the Office of Civil Defense,

Department of Defense. This research includes a basic investigation of chemical changes involved in oxidative breakdown of fats in cereal products, intended to provide a foundation for development of measurements of food deterioration that will be useful in surveillance of food supplies. New insight gained in the mechanism of autoxidation of methyl linoleate is providing guidance to what actually occurs during oxidation of fats in foods. Results being obtained now at low oxidation levels and at ordinary temperatures are more appropriate models of changes that occur in foods than were the accelerated aging tests used in the past. The primary products of lipid autoxidation, such as aldehydes, are subject to further reactions, for example, oxidation to acids and condensation to trioxolane. Therefore, the primary products are unsatisfactory indicators of the progress of rancidification, and their concentration in the gas space of a food container is not a reliable objective measure of rancidity. Unreactive compounds, such as saturated hydrocarbons, carbon dioxide, and carbon monoxide, which are also products of autoxidation, may furnish even better objective tests, if their development is found to be well correlated with rancidity as determined by organoleptic means.

### C. Color, Texture and Other Quality Factors

1. Color. Contract research at Oregon State University is conducted to determine the nature of the color substances in wheat bran and aleurone tissues. A series of phenolic compounds in free and combined state have been found that appear to be the coloring material of the bran. Spectroscopic investigation revealed simple phenolic patterns consisting of mono- and disubstituted aldehydes and acids. The polymeric materials belong to a chemical class known as phlobaphenes. Extracts of red brans from three genetically pure wheat varieties, which were different in intensity of red color, showed differences in both the amount and the nature of isolated materials. These basic studies are expected to provide a foundation for developing decolorizing procedures to use in making whole kernel products and products derived from wheat bran and other milling fractions.

We are also conducting in-house research on bran pigments in connection with studies of proteins that can be extracted from bran. In bran there are two distinct types of pigments, one yellow and one brown. The brown is more closely bound to protein. There is also a clear distinction between protein that adsorbs or binds brown pigment and protein that doesn't. We are now attempting to isolate reasonably pure protein of the type that binds brown pigment in order to determine the nature of the binding.

2. Dough Characteristics. The properties of bread doughs change with mixing; we are studying the chemical reactions involved. The sulfhydryl groups (free sulfur hydrogen terminal groups) of flour proteins appear to be more important to the physical properties and baking behavior of doughs than their low concentration would lead us to expect. Sulfhydryl contents decrease when flours or doughs are matured by oxidation, but sulfhydryl groups must be present during dough mixing if the dough is to have normal elasticity and



extensibility. Doughs mixed in air, so that some sulfhydryl groups are oxidized, differ in physical properties from doughs mixed in nitrogen. Earlier it was believed that the sulfhydryl level did not change significantly when doughs were mixed under nitrogen so that oxidation was prevented. However, we have found that the titratable sulfhydryl groups decrease rapidly in the first two or three minutes of mixing and then return to the original level in 5 to 10 minutes. If the free lipids are removed from the flours by solvent extraction, the initial rapid decrease is eliminated. With longer mixing, sulfhydryl content increases slowly in all flours. Mechanical action in dough mixing is required for such increases, which have been observed to be as much as 50% above the initial flour level. Formation or unmasking of sulfhydryl groups may be the explanation of the reversals in the effects of chemical oxidizing agents and sulfhydryl blocking reagents on the freeing of sulfhydryl groups by dough mixing. Hydrogen sulfide has been detected in the nitrogen atmosphere above doughs when they are protected from air during mixing. The amount of hydrogen sulfide evolved depends upon the mechanical energy applied to the doughs. No hydrogen sulfide has been released by stirring flour water suspensions. If sulfhydryl blocking reagents (iodate or bromate or yeast) are added to the doughs, the amount of hydrogen sulfide released is smaller.

In contract research conducted at Washington State University, the fate of extractable proteins is being followed by use of radioactive tracers. By growing wheat in the presence of radioactive carbon dioxide, a certain amount of radioactivity is incorporated into the wheat kernel and the flour that is obtained from it. Examination of the wheat protein by starch gel electrophoresis indicates that dough mixing transfers radioactive tracers from the slow-moving proteins to the fast-moving proteins, which indicates that protein has been degraded in the mixing process. Possibly fast-moving proteins (albumin or globulin) are released from a state of being bound to slow-moving proteins (gliadins or glutenin). The binding could very well be by disulfide bonds which can be ruptured by mixing.

Basic studies on the solubility of wheat gluten proteins in aqueous systems and on correlations between protein components and baking quality of flours are being conducted under a P.L. 480 grant at the National Center for Scientific Research at Montpellier, France. Three varieties of wheat flour that differ in baking quality have been tested. Although the maximum solubility in water of the gluteins was approximately the same for all three flours, the rate of solubilization seemed to correlate with the baking quality--the greater the rate of solubilization the better the baking quality.

At the British Baking Industries Research Association in Chorleywood, England, the effects upon baking quality of variation in wheat flour lipid are being investigated under a P.L. 480 grant. The extractability of lipids from flours from two wheat varieties having different protein contents and different baking quality was studied. Differential extraction of various lipid components with solvent is affected by the amount of water present. Certain binding sites of lipid-to-protein appear to have higher affinity than others.

In a strong wheat flour, 67% of the lipid was associated with gluten proteins, 22% with starch, and 11% with water solubles. Chemical treatment of flour with an agent which causes loss of cohesion in gluten did not change the yields of free and bound lipid.

In the absence of salt, a fat added in dough making increases the binding affinity of phospholipids. With optimum oxidation of dough, addition of fat improved the baking quality still more. The added fat had no effect on the gas retained by a dough at 80° F. or lost at 130° F., and the fat exerted its improving effect in the absence of yeast when glucono-delta-lactone was used as a raising agent. In a study of baking processes that occur in the oven, no evidence was found that fat affects denaturation of protein. In addition to improving loaf rise, fat present in the dough was responsible for a more rapid loss of moisture in the crust, compared with loaves made without added fat.

Interactions of nonfat dry milk and  $\alpha_s$ -casein on dough and bread qualities are being investigated by employing a brew fermentation and a Do-Corder to simulate continuous mixing. With brews containing no flour or only 10% flour, 6% nonfat dry milk consistently caused decreases in crumb score and loaf volume, as expected. When 0.28%  $\alpha_s$ -casein replaced 4% of the dry milk, adverse effects were no longer observed. A volume increase usually was obtained when the  $\alpha_s$ -casein was hydrated separately from the brew and added at the dough-mixing stage. In contrast, with brews containing 30% flour,  $\alpha_s$ -casein in the brew had no effect unless hydrated separately, in which case it gave a small volume decrease. The decrease, however, was not as great as that caused by an equivalent amount of nonfat dry milk. From these results it appears that the action of a protein-splitting enzyme in the flour on  $\alpha_s$ -casein from the nonfat dry milk may be of only minor importance to continuous-mix bread production. The commercial procedures in continuous-mix bread making are trending towards a higher percentage of flours in brews (as much as 70%). The relatively poor behavior of either isolated  $\alpha_s$ -casein or nonfat dry milk in brews containing only 30% of the formula flour indicates that a problem exists that should be studied further.

3. Dough Rheology. The rheological properties of wheat flour doughs play a major role in determining the properties of baked products, so numerous attempts have been made to find rheological testing procedures that correlate with baking quality. Such instruments as the Farinograph and Extensograph produce characteristic curves that correlate with various aspects of flour quality and thus help to improve control over the operation of mills or bakeries. But these instruments are empirical in principle and they measure a complex interaction of several factors, so changes in baking technology (including high-speed mixing, mechanical dough development, continuous processes, etc.) as well as the varying reactions of chemical improvers complicate the problem. Furthermore, sample geometry and loading patterns are not well defined, and these factors influence the experimental results to the extent that they cannot be meaningfully compared with other measurements.



The aim of a fundamental study of dough rheology is to describe macroscopic rheological phenomena in terms of the underlying molecular structure in a bread dough. To do this, we try to observe fundamental stress-strain relationships and compare them with the stress-strain predicted from an assumed molecular architecture. Stress-strain relationships can be measured in terms of elongation or resistance to shear forces. It is difficult to maintain constant sample geometry for measurements of elongation because of gravitational forces on the sample, and it is impossible to achieve pure elongation without introducing some form of shear. Non-uniformities of stress throughout the sample are a further disadvantage. Shear measurements have the advantage of maintaining constant sample geometry so that, for all equivalent points in the material, stress and strain are homogeneous. In dough rheology, a static approach has been used almost exclusively, but a complete description of rheological behavior must be made over a wide time scale. To cover this, dynamic measurements are preferable but because of instrument complexity, they have generally been avoided. The availability of electronic apparatus and electromechanical transducers to refine measurements and computers to solve mathematical equations of physical systems have been responsible for the recent advances in rheological theories. By necessity, the mathematics are very complex. It is only with a very sophisticated level of rheological inquiry that the complexities of dough rheology are likely to be delineated.

Basic investigations on dough rheology are being conducted by contract at Stanford Research Institute in Menlo Park, California, and by P.L. 480 fund grants to the Rheological Laboratory of the Israel Institute of Technology in Haifa and to the Bread Research Institute of Australia in North Ryde, New South Wales.

In Australia a suitable research instrument has been designed and constructed. A dough sample is sheared by application of a sinusoidal varying force that causes the inner of two concentric cylinders to oscillate. Dough samples in the annular space between the cylinders resist the oscillation, and measurement of this resistance provides data on the viscoelastic properties of the samples. When very small strains are induced, the physical properties of the dough are not influenced by deforming stresses, that is, the behavior is linear. At larger strains, however, the effects are not linear. In previous work of this type, only larger strains were imposed, hence development of a mathematical model was seriously hampered by the nonlinearity of the stress-strain formulas. The complicated effects of temperature, water absorption, and oscillation frequency on viscoelastic functions is being investigated. Preliminary investigation of oxidative improvers indicate that they affect the elastic property much more than the viscous property. This interesting lead will be pursued.

In closely related studies at Stanford Research Institute, large deformation and failure properties are being investigated by pulling dough rings on an Instron tester. Rings with a horizontal pull are less satisfactory than those with a vertical pull, because of the nonuniform friction between horizontal



dough rings and the tray that supports them. The vertical method too, has errors which are introduced by the lack of free slippage over the hooks holding the rings and the sag of the rings under their own weight, but these problems can be largely corrected for. Stress-strain data obtained early in this work showed considerable scatter, but improvements are contemplated in which the dough rings will be immersed in a fluorocarbon or silicone oil of density equivalent to that of dough so that buoyant effects will eliminate sagging of specimens. Dynamic methods for testing linear viscoelastic behavior are also being studied, and equipment is being designed and constructed similar to that available for other types of polymeric materials. Dough differs from many viscoelastic materials in that it can be tested over only a very limited temperature range; other substances can be tested over a wide temperature range at limited frequencies of dynamic stress. To obtain equivalent information for doughs, the measuring instrument must have a much wider frequency range.

In the project in Israel, very rapid measurements are made of tensile stress and strain relationships, relaxation times, and elastic recovery behavior in dough specimens taken at intervals during the dough-mixing cycle. Instruments for rheological measurement were redesigned, then data were obtained to evaluate possible correlations with Farinograph and Brabender Extensograph measurements. Measurements that separated the elastic and viscous components of dough deformation led to a revision of the tentative mathematical model of wheat flour dough. Data will now be obtained on flours from four varieties of wheat supplied from the United States.

The group in Israel also designed new instruments for measurement of viscoelastic properties of dough. One of them, a constant-velocity extensometer, measures dough at high stresses and rates of strain for comparison with the Brabender Extensograph. With this equipment, viscous and elastic deformations can be determined over a much wider range of loading times than usual; these data can be related to the decay of elasticity in dough. In order to widen the range of viscoelastic properties of dough, additives known to affect mechanical properties of dough have been used. Although knowledge of dough properties has been materially advanced in this work, the correlations between these properties and the baking quality of flours are still somewhat obscure. A good foundation is being laid, however, for an ultimate solution of the rheological problems of dough mixing and baking.

#### D. Microbiology and Toxicology

1. Microbiology of Wheat Food Products. The heat of baking destroys most of the contaminating microorganisms in conventional bakery foods, but when a flour is to be used in foods such as meat pies, soups, and biscuits intended for frozen or refrigerated storage, a low microbial population is highly desirable. If such products are improperly handled, spoilage may result. Contract research on means for reducing microbial contamination of milled flour has been concluded at the American Institute of Baking in Chicago. Four different types of microorganisms (Bacillus subtilis, Aspergillus flavus,

Staphylococcus aureus, and Escherichia coli) were used, individually, to inoculate flour at the rate of 1 million per gram. Frozen pie dough and soup prepared from these flours were tested at -3° F. and at 20° F. The higher temperature represents grossly unsatisfactory storage conditions for frozen products. Biscuit dough was stored at 37° F., representing good storage temperature, and at 37° F. cycled once a day to 70° F. for 6 hours to represent grossly unsatisfactory conditions of storage. At the unsatisfactory storage temperatures, pie dough became rancid; soup underwent a drastic change in consistency and browned excessively; refrigerated biscuits lost volume when baked, the crumb became gummy and gray, and an off-odor developed. Stability of frozen products was very materially reduced by the use of inoculated flour as compared with uninoculated flour. Inoculation did not materially affect stability of the biscuit doughs, but the storage defects were drastic even with flour that had not been inoculated. Propylene oxide can be used to reduce microbial contamination, but a more desirable control of micro-organisms was found to be a 45-minute heat treatment of flour at 130° C. Heating at this temperature is nearly as effective as treatment with propylene oxide at levels of 100 parts per million. The cost of propylene oxide, its flammability, and the possibility of an odorous residue lead to the conclusion that heat treatment of flour is better than the chemical treatment.

#### E. Technology--Process and Product Development

1. Bulgur, WURLD Wheat, and Related Products. The capacity for food production in North America that exceeds local population needs is a weapon for combating hunger in the developing nations of the world. The surplus conditions are expected to continue for two decades or more, which may provide sufficient time for agricultural advances that hopefully will make the developing nations more self-sufficient. The North American surplus productive capacity is for wheat, but most of the food-deficient areas of the world do not have a tradition of eating wheat or wheat products.

A new product developed at the Western Lab, WURLD wheat, may be a form that is acceptable in the areas of greatest need. It is a whole kernel or cracked kernel product from which the bran has been removed by lye-peeling. A 2500-lb. lot of WURLD wheat shipped to Hong Kong over a year ago for pilot acceptance tests by voluntary welfare agencies was sufficiently successful to warrant further acceptability and marketability tests in Hong Kong. Pilot plant operations were scaled up to produce at a level of about 200 lbs. per hour. Now a 3-ton lot of WURLD wheat has been sent to Hong Kong for testing under the auspices of the Church World Service. We hope to get data on commercial marketability as well as consumer acceptance from this second trial. Two companies have conferred with us about the WURLD wheat peeling operation; one company can produce WURLD wheat at a rate of 2 tons per hour, the other at 25 tons per hour. Stability studies of WURLD wheat indicate that it has sufficient shelf life to enter distribution channels like those used for bulgur.

Both gun-puffed bulgur and WURLD wheat have a pleasant mildly toasted taste and they are easily chewed. WURLD wheat puffed in hot air is more tender and

friable than the product obtained from bulgur, and it is much better for use in confectionery and bakery products as well as in convenience products requiring rapid uptake of water.

WORLD wheat can carry a high level of high-quality protein when it is coated with soy flour or high-protein fractions obtained from soy beans. Coatings are steamed in place on whole or cracked kernels and then dried. The coating becomes very firmly attached; it does not slough off even when cooked in excess water. We have also added protein to bulgur, rolled wheat, rolled bulgur and rolled WORLD wheat in this way. A protein-enriched rolled wheat or rolled bulgur can be instantly reconstituted into a highly nutritious gruel by mixing it with boiling water. As such it can be a health giving food for undernourished children in regions where protein deficiency abounds.

Stabilities of bulgur wafers and of a line of food adjuncts to make bulgur wafers more palatable are being studied under a purchase contract at Oregon State University in Corvallis. This research is supported by transfer of funds from the Office of Civil Defense, Department of Defense. After the wafers had been stored 40 months, taste panels preferred those made from bulgur that had received an atmospheric rather than a pressure cook and had been compounded with malt syrup rather than corn syrup, and packed in an atmosphere of nitrogen instead of air and stored at 70° F. or lower. Analyses of headspace gas show that more carbon monoxide and carbon dioxide are produced by wafers packed without nitrogen replacement of the air and stored at higher temperatures, and the amount of oxygen absorbed or chemically reacted is greater.

Twelve adjuncts for use with bulgur wafer in rations stocked in fallout shelters have been in storage for 2-1/2 years. Most of them benefit from being packed in a nitrogen atmosphere; only beef soup mix and dry strawberry spread mix seem to survive better in air. Apple topping and wild cherry icing mixes show no differences. In-package desiccant was helpful in maintaining quality in chicken soup, chocolate pudding, and curry sauce mixes. As expected, the lower storage temperatures were generally advantageous. Carbon dioxide in headspace gas paralleled deteriorative change in that more carbon dioxide was found in samples stored at higher temperatures. However, the liberation of carbon dioxide cannot be measured in samples containing an in-package desiccant because it is absorbed by the desiccant. Carbon monoxide measurement, however, may be useful as compositional data that parallel deteriorative processes. Under carefully controlled and reproducible conditions, the measurement of carbon dioxide and carbon monoxide may be useful in determining residual storage life of canned dry products.

2. New Wheat-Based Foods. Using inexpensive dry milling methods, we have concentrated protein of high biological value from millfeeds. In this way, sizable proportions of these low-cost materials can be recovered for food as well as for high-quality feed ingredients. Application of these processes could result in greater financial returns to millers, offer relief of pressure against high prices for flour, and lead to recovery of valuable protein



for use in developing countries overseas. By dry milling and sifting, protein concentrates with low-fiber content have been prepared from wheat millfeeds, coarse bran, fine bran and shorts--byproducts now used as animal feed. Starch and total sugars, as well as protein, were concentrated in the fine flour-like fraction that passed a 7XX bolting cloth; fiber, ash, and pentosan contents were reduced; fat content remained relatively unchanged. Adjustment of moisture content of the millfeeds before milling greatly affected the yield of flour-like product as well as its protein concentration. Highest yields were obtained at lowest moisture levels, but for most desirable composition and ease in processing conditions, the moisture range of 9 to 11% appears most promising. At this moisture content, 20 to 30% of bran fractions can be recovered as protein concentrate and 50% of shorts. These concentrates have 2 to 4% fiber and, therefore, can be used as human food. Amino acid analysis and protein efficiency ratio evaluations with laboratory animals show that the concentrate from shorts is of good nutritional quality.

Protein concentrates from shorts have been formulated with farina, corn meal, nonfat dry milk solids and soy flour for use as simple gruel products that are palatable and highly nutritious. A new process was derived to prepare protein concentrates from flour by incorporation of soluble soy protein and vegetable oils which modified the gluten recovery from a water slurry. The method has an advantage over conventional wet milling in that a starch by-product can be easily separated from the dispersed gluten and recovered. Simplification of the separation of starch and protein from flour should reduce the cost of this process.

High-protein wheat flours can be converted to milk-like beverages that have bland flavor and light color. They are easily dispersed in water. To improve dispersibility flour or the protein concentrates are digested with starch and protein-splitting enzymes under controlled conditions. The products can be used as beverages and gruels and should be useful particularly in enriching diets of infants and small children in regions of protein deficiency.

3. Carrying Capacity of Hard Red Winter Wheat. To provide information that may lead to wider markets for surplus United States hard red winter wheats, we are investigating the capacity of these wheats to carry the soft low-protein European wheats in the making of high-quality yeast-raised bread. Research on blending characteristics is conducted, by contract at Kansas State University, with samples of soft wheats procured from Germany, England, Sweden, The Netherlands, Austria, France, and Belgium and samples of hard red winter and spring wheats from the United States and Canada. European wheats are being tested for milling, blending, and baking characteristics. In addition, two South African soft wheats have been used in the tests, because their resemblance to European soft wheats provides a better exploratory experiment than would have been possible with United States soft wheats. Even relatively small additions of higher protein hard wheats from the United States improved baking characteristics of soft wheats in regard to absorption, mixing tolerance, loaf volume, and crumb texture. U.S. winter wheats at two

protein levels (10.0 and 11.3%) produced some improvement, but North American spring wheat of still higher protein level gave better results.

Flours from Swedish and Belgian wheats required no malt in the bread formulas, indicating the presence of sprouted or immature wheat--a major problem to European flour mills. High humidity in grain at time of harvest frequently allows some of the wheat to sprout during harvest and storage.

4. Malting Technology. We have discovered that agitating a bed of wheat or barley during the steeping process inhibits sprouting but not enzyme formation. Malting capacity for a given volume of equipment can be increased 50% by agitation, and loss of dry material from the malted wheat is less because no rootlets develop. Such factors as lack of oxygen in the steeping solution, effect of elevated temperatures, and the production of a water barrier beneath the kernel epidermis were found not to be responsible for the sprout inhibition. In normal steeping, a growth-inhibiting substance is eluted from the hulls of wheat and barley, and more of it is produced under agitation. We suggest that diffusion of this substance into the kernel is greater as a result of the agitation. Another hypothesis for the difference between still and agitative steeping is that mechanical damage to the germ may result from agitation.

Contract research is being conducted at the University of Minnesota in St. Paul to study the changes in proteins of wheat during malting. Progress has been made in developing methods for extracting and fractionating nitrogenous constituents of ungerminated and germinated wheat, and a preliminary confirmation of the expected increase of protein efficiency ratio as a result of germination has been obtained. The prospects are encouraging for development of new and improved wheat food products with enhanced nutritive properties.

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## AREA NO. 2. WHEAT UTILIZATION - FEED

Problem. Wheat can now compete with feed grains as animal feed because new legislation on wheat pricing makes it economical. In the last two years the use of wheat for feed increased to nearly 100 million bushels per year, more than twice the amount used in any other recent year. Unfortunately, wheat has certain performance drawbacks as a feed. Research to improve the feeding quality of wheat would greatly benefit both growers and feeders in wheat-producing areas, since it would place this grain in a more competitive position as compared with other grains.

Millfeeds are not used extensively in modern poultry and swine rations because the high fiber content cannot be tolerated in high-energy rations. If inexpensive ways of separating low-fiber, high-protein fractions from millfeeds are developed, these new materials can be used as protein and energy sources for non-ruminant diets, and the overall value of milling byproducts will be increased. Flour production is expected to increase in the near future to reflect the demands of our increasing domestic population and of the new export markets which are developing. More milling will result, of course, in more millfeeds. If these millfeeds cannot be utilized efficiently and effectively, the price for flour will have to increase to carry the economic burden.

Meat production, particularly poultry, is increasing rapidly in Japan and the European Economic Community where modern efficient methods have been introduced. This development depresses the opportunity for exporting poultry and other meats into these important trade areas, but it offers an increasing opportunity to sell feeds. Upgrading of wheat millfeeds through utilization research will increase our export markets.

### USDA AND COOPERATIVE PROGRAM

Research at Albany, California, on utilization of wheat seeks to develop new processes to convert milling byproducts into high value feeds and to modify whole wheat so that it is more economical for use as a feed grain. Research on barley is also conducted.

The Federal program of research in this area totals 2.9 scientist man-years, of which 1.4 are assigned to investigations on chemical composition and physical properties and 1.5 are assigned to technology--process and product development.

### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3 scientist man-years is devoted to this area of research.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

### A. Chemical Composition and Physical Properties

1. Wheat Proteins. We are improving the methods for amino acid analysis of wheat products. The hydrolysis step is the major cause of variability of analytical results. Some amino acids (e.g., threonine, serine, cystine and methionine) are partially destroyed during hydrolysis. Other amino acids are liberated very slowly and require excessively prolonged hydrolysis (e.g., valine and isoleucine). The relative rates of destruction of labile amino acids and liberation of other amino acids are uniform for food or feed products but not for pure isolated proteins. A systematic study was made using an accelerated system with an automatic amino acid analyzer to determine effects of time of hydrolysis of wheat and wheat bran on apparent amino acid composition. Correction factors were calculated to permit precise determination, from a single 24-hour hydrolysate, of all common amino acids of wheat except cystine, methionine and tryptophane. A second hydrolysis procedure is used to determine cystine and methionine.

2. Non-protein Components. Progress has been made on methods of analysis for nondigestible holocellulose and lignin in millfeeds. The large amount of starch present interferes with the analysis. We have found it is better to remove most but not all the starch by enzymes and then determine the amount of starch remaining. The procedure is simple compared with enzymic removal of all of the starch. We are also testing and developing simplified methods for analyzing lipids in samples of wheat and mill fractions. Test material is supplied by the Technical Subcommittee on Millfeeds of the Millers National Federation.

### B. Technology--Process and Product Development

1. Improved Feeds from Milling Byproducts. Efficient utilization of millfeeds is a major problem facing millers, growers, and a hungry world. Nearly 28% of whole grain wheat is recovered as non-flour byproducts during milling of white flour. These millfeeds have been sold as feeds, and only recently has an intensive research program been undertaken to upgrade both their dollar and nutritional value. Milling byproducts contain important nutrients at levels of concentration even higher than in the milled flour, but because of general variability of quality and condition, they cannot compete for use in mixed feeds with other sources of protein, carbohydrate and other nutrients. The unrealized feed values in mill byproducts adversely affect milling profits and are reflected in higher flour prices as well as in pressure to reduce wheat prices to growers.

We are preparing protein concentrates with low fiber content from wheat millfeeds, coarse bran, fine bran, and shorts by dry milling and sifting. In addition to protein, much of the starch and total sugars from these feed fractions are also concentrated in the fine flourlike fractions. Fiber, ash and pentosan contents, on the other hand, are reduced. Moisture content



of millfeeds before milling greatly affects yield of protein concentrate as well as the concentration of the protein. Highest yields were obtained with low moisture level but, from the standpoint of desirable composition and ease of processing, remilling the millfeed fractions at a moisture range of 9-11% appears most promising. Yields of 20-30% of low-fiber protein concentrate are possible with bran fractions; up to 50% yields are possible with shorts. Fiber content of these products ranges from 2-4%, so they have utility in mixed feeds for non-ruminants and also for human food. Amino acid analyses and protein efficiency ratios show that the concentrate from shorts is of good nutritional quality.

2. Improved Use of Wheat for Feeds. Experiments are underway to improve the nutritional quality of wheats by simple processing treatments. Preliminary information has been obtained on pressure cooking of wheat at various steam pressures, with and without added acid, and on hot-air puffing of whole wheat. Data obtained from tests in an artificial rumen indicate that nutritive value is improved, both by cooking and by puffing. If these data can be verified with large animals under usual fattening conditions, they will indicate that costs of meat production can be reduced significantly.

We are supporting research with P.L. 480 funds at Cambridge University in England to develop rapid chemical methods that can be applied during processing of wheat and wheat products for assay of the biological value of wheat proteins. Our work on improvement in nutritive value of wheat, both for human and for animal feeding, by controlled moisture-heat treatments stems from the observations of this project. Careful cooking and drying of wheat improves its protein efficiency ratio, although other protein quality measurements are less affected. But careful control in processing cereal foods and feeds is necessary, because overheating can impair nutritive quality. It is apparent that the major effects of cooking are changes in carbohydrate and other nonprotein wheat components. Although a rapid chemical method for assay of biological value of wheat proteins has not been developed in this project, a number of useful leads for further research have been obtained.

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1/ Research supported by P.L. 480 funds.

### AREA NO. 3. RICE UTILIZATION - FOOD

Problem. Limitations on U.S. rice acreage are being relaxed because of the increasing need for rice to export into food-short areas, particularly Southeast Asia. Polished rice is a relatively expensive source of nutrients because its protein, although high in quality, is only about 5% to 7% of the kernel, and it is almost devoid of vitamins. The need is for new and improved food products with better nutritive quality and produced at minimum increase in cost. Such products must be easy to prepare; have good texture, flavor, and appearance; and be economical to manufacture. Also needed are drastically improved milling methods to increase economic returns to growers and millers so that cost to consumers will not increase. Detailed knowledge of chemical composition and physical properties as related to processing is needed to guide the developments in milling, processing, and development of products that meet the growing export demand.

#### USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, basic and applied research on rice is conducted at Albany, California. Basic studies are concentrating on rice proteins. Process research is underway on debranning of brown rice by lye-peeling, parboiling of brown and under-milled rice, new methods to produce quick-cooking forms having better flavor and texture, and conversion of high-protein flours into beverage products especially suitable for infant feeding overseas. Cooperative studies with industry and the University of California are conducted on improved methods for drying rice.

The Federal program of research in this area totals 2.4 professional man-years. Of this number 1.2 are assigned to chemical composition and physical properties and 1.2 to technology--process and product development.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition and Physical Properties

1. Rice Proteins. Studies in the past few years have begun to supply knowledge on the fundamental makeup of rice proteins. We are continuing these studies in order to establish a firmer base on which to develop high-protein rice foods. Extracts of rice prolamines (proteins which are soluble in 60-80% alcohol) have been resolved into seven components, one of which is predominant and four very minor. All of these components



differ distinctly from the components of globulin proteins (proteins which are soluble in a salt solution). The prolamine constituents are being separated, in order to determine individual amino acid compositions.

Isolated rice globulins have been segregated into single components on the basis of solubility differences. One protein component comprising 40-45% of rice globulins has been partially characterized. It has a molecular weight of about 25,000 and is rich in arginine and glutamic acid; it contains considerable cystine and methionine and is almost devoid of lysine and histidine. The outer layers of rice endosperm (see discussion on high-protein rice flours, Paragraph B. 1. below) contain higher proportions of soluble proteins; a decreasing gradient in biological value of protein may exist as well as a decreasing gradient in concentration of protein.

## B. Technology--Process and Product Development

1. Improved Rice Products. Abrasive milling of flour from surface layers of milled rice in commercial mills is possible with all types of rice. Flours with about double the protein content of the original kernels have been produced. Dry powders that are easily dispersible in cold water to form a milk-like beverage have been made from high-protein flours of both regular and parboiled rice by two processes, one involving partial hydrolysis of protein by pepsin and the other using amylase plus pepsin to hydrolyze the starch as well as the protein. Simple mixing of the dry solids with water produces beverage foods of milk-like consistency.

High-protein flour obtained by deep milling of parboiled rice is practically equivalent in protein efficiency value to similar flour obtained from white milled rice. This indicates that the parboiling process does not significantly reduce the nutritive quality of the rice protein. In addition to doubling the protein content, deep milling provides several-fold increases in the vitamins thiamine and riboflavin and in fats and minerals.

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#### AREA NO. 4. FORAGE UTILIZATION - FEED

Problem. The demand for livestock in the United States will increase 45% by 1975. Since forage crops constitute the major feedstuff for ruminant animals, the demand for forages will increase accordingly. In addition, there is an increasing demand for processed forages in European and Asiatic markets. Fresh forage crops are the richest natural source of many nutrients for farm animals. Forages, however, are preserved so inefficiently by hay-making and ensiling that 10 to 50% of the dry weight and much larger fractions of the most valuable nutrients are lost before the animals eat them. Dehydration is now the only practical means of producing products of high nutritional value in a form usable in manufactured feeds and supplements. Poultry and swine producers are aware of the value of dehydrated forage, but restrict their use of it because of its high fiber and growth-inhibitor content. There is evidence that certain unidentified growth factors are lost, at least partially, during the dehydration process as presently carried out. The livestock breeder needs forage products tailored to specific animals, and the forage producer must adapt to his needs to sell.

Basic and applied utilization research are necessary to produce: (1) high-protein, low-fiber feeds rich in unidentified growth factors designed for use by non-ruminant animals; (2) fiber products which have been cheaply treated to make them easily digestible for ruminants; (3) growth-stimulating supplements for ruminants, derived from the biologically active fiber-digestion factors and growth-promoting factors in forage. New products should be adaptable to mechanical feeding. Improved uses will encourage farmers to put high-value land into forage crops.

#### USDA AND COOPERATIVE PROGRAM

Current research in the Western Utilization Research and Development Division includes both basic and applied studies on all forages used or potentially usable for off-the-farm processing. The research is conducted at the Division headquarters at Albany, California; under contracts at Berkeley, California, Lincoln, Nebraska, and Athens, Georgia; and Under P.L. 480 grant programs in Scotland and Italy. Basic compositional studies deal with the potent estrogen, coumestrol (discovered by Department scientists), and other phenolic compounds present in forage legumes. The value of coumestrol-rich alfalfa as a growth stimulant for ruminants is being studied in cooperation with Oregon State University. The mechanical separation of leaf from stem of alfalfa is being studied with financial support from the Department of Agriculture and Inspection of the State of Nebraska and the cooperation of several experiment stations and commercial processors and users of forages. Also under study are biologically active forage constituents (such as the chick-growth-promoting factor in forage juices and alfalfa saponins which depress chick growth), organic acids of alfalfa, non-protein nitrogen compounds of alfalfa, and the mechanism of action of forage antioxidants.



Processing of forages by "wet" (juicing) and "dry" (turbomilling and air classification) methods is being investigated. The effects of dehydration conditions on losses of carotene and xanthophyll are being studied.

The Federal program of research in this area totals 7.4 scientist man-years, including one scientist whose salary is provided by the Department of Agriculture and Inspection, State of Nebraska, and contract research equivalent to 2.0 scientist man-years per year. Of this number 4.5 are assigned to chemical composition and physical properties and 2.9 to technology--process and product development. In addition the Division sponsors, under P.L. 480, two research projects on forage composition.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 10 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition and Physical Properties

1. Forage Composition. Because of their association with biological activity, phenolic compounds of alfalfa and other forages are being isolated and identified. Two compounds from ladino clover, in addition to those reported earlier, have been characterized as 7-hydroxycoumarin and 7,7'-dihydroxy-6,8'-bicoumarinyl. The structure of two previously unknown compounds from alfalfa has been confirmed by unequivocal synthesis as 7-hydroxy-11,12-dimethoxycoumestan and 7,12-dihydroxy-11-methoxycoumestan. A series of 20 coumestans isomeric with coumestrol and its mono- and dimethoxy derivatives have been synthesized for chemical, physical, and biological comparison with coumestrol.

Several previously unidentified acids of alfalfa have been tentatively identified as monosodium and monoethyl derivatives of dicarboxylic acid. During the study of previously known constituents by means of new analytical methods, new unidentified acids have been detected. Analyses of holocellulose and lignin by several procedures on a series of alfalfa samples have been completed. We will determine which of these procedures correlates best with metabolizable energy value for chicks.

Research on a method for analyzing xanthophyll has led to a new cold saponification step which eliminates the chlorophyll error in methods previously available. The new procedure is simplified yet accurate compared with other methods. It has been adopted for a collaborative evaluation by industry and should give the feed formulator confidence in employing alfalfa meal as the source of xanthophyll pigmenters in poultry feed. In studies of dehydration, the effects of moisture of meal and inlet and outlet temperatures on the xanthophyll content and extent of carotene isomerization are being examined. As much as 50% of the xanthophyll content of fresh alfalfa may be destroyed by improper dehydration procedures.

In cooperative work with the Nutrition Department at the University of California, evidence has been found for a growth factor in dehydrated alfalfa. By adding 10% of dehydrated alfalfa to the diet of guinea pigs, improvement in growth of up to 50 g. over a 3-week assay period was obtained.

The factor(s) appears to be distinct from all known vitamins, minerals, amino acids, and other nutrients and appears to be organic in nature, since it is not found in the ash of alfalfa. Studies are now in progress to define the properties and nature of this unidentified factor(s).

2. Interactions of Forage Antioxidants. Pigments, vitamin E, lipids and related substances provide much of the biological value of alfalfa, but they are subject to oxidative deterioration. Contract research on chemical changes of alfalfa lipids is being conducted at the University of California at Berkeley to provide basic information that could lead to stabilization of alfalfa and other forage products. The lipid composition of fresh alfalfa and changes that occurred immediately after cutting were determined. Beta-carotene content dropped rapidly immediately after cutting. Linolenic acid, the major fatty acid in alfalfa lipids, did not decrease until after the first 24 hours but then rapidly decreased to less than 50% of its original value in 48 hours. Linolenic acid is converted to products that have been extremely difficult to extract from alfalfa. Some minor increases occurred in palmitic, stearic, and oleic acid, but other fatty acids did not change. Since about 90% of the fatty acid present in alfalfa galactolipids is linolenic acid, substantial changes must take place in the galactolipids as harvested alfalfa is field cured over a two- to several-day period. Monogalactolipids are more prone to change than digalactolipids. Galactolipids affect antioxidant activity, so experiments were initiated to determine the effect of antioxidants on the rate of disappearance of beta-carotene and linolenic acid in alfalfa. In addition, the natural antioxidants from alfalfa were tested on other oxidizable lipids. Monogalactolipid was effective as an antioxidant for menhaden oil but showed no antioxidant activity for squalene. Digalactolipid was not effective as an antioxidant. The monogalactolipid content of alfalfa is about twice the digalactolipid content.

Under a P.L. 480 grant, research at the Experiment Station for Practical Agriculture in Milan, Italy, is underway on non-tocopherol reducing substances in alfalfa that may be involved in the natural antioxidant activity of alfalfa products. Natural antioxidants exist in alfalfa with activity that is several times as great as the antioxidant activity of tocopherol. Methods for separation, identification, and assay were developed that reduced to a minimum the alteration and destruction of the compounds during the laboratory procedures. Qualitative recognition and semiquantitative evaluation of the substances were carried out. At least four reducing compounds, whose evolution was followed during the growth of the plant, were isolated. During regrowth of alfalfa after cutting, one of the compounds increased in large amounts during the first vegetative preflowering stage. Another compound, absent during the first week of vegetation, resolved into three distinct compounds, one of which predominated over the other to become four or

five times more abundant by the time of flowering. During the winter, no appreciable amounts of these reducing compounds could be identified, either in hibernating plants in the open field or in those vegetating in greenhouses. These non-tocopherol reducing agents are probably formed during the plant's period of highest photosynthetic activity. Chromatographic RF value provided a tentative identification of one of the compounds.

3. Structure of Alfalfa Polysaccharides. Research is being conducted under P.L. 480 funds at Edinburgh University in Scotland to determine the nature of alfalfa polysaccharides and to investigate enzyme systems that may be helpful in structural analysis of the polysaccharides and components associated with them. The amylase activity of alfalfa seedling extracts is very similar to that of malt alpha amylase. Amylase can split large molecules made up of 6-carbon glucose. The extracts from alfalfa also show enzyme activity in hydrolyzing large compounds made of the 5-carbon sugar xylose. The main hemicellulosic component of alfalfa stems has been characterized as to its constituent carbohydrates. A similar component is present in the alfalfa leaf, and structural examination has been conducted in sufficient detail to establish its essential identity to the stem hemicellulose. Sufficient quantities of oligosaccharides and D-galacturonic acid were prepared for a study to be made of the enzyme activities that will hydrolyze these compounds.

#### B. Technology--Process and Product Development

1. Improved Feeds from Forages. Preliminary tests with high-estrogen forages or extracts prepared from them indicate that naturally occurring plant estrogens have growth-promoting effects on sheep. Four feeding trials with lambs fed varying amounts of alfalfa coumestrol were conducted at Oregon State University on a cooperative basis. Tests were made with crude alfalfa meals, acetone extracts of different coumestrol potencies, and with isolated coumestrol. Except with the isolated coumestrol, a trend toward positive growth response to elevated coumestrol levels was obtained with wether lambs but not with ewes. Estrogenic responses were observed in animals fed the higher levels of coumestrol. In sensory tests, lamb roasts from animals fed high coumestrol diets consistently scored higher in tenderness and juciness.

A study was conducted cooperatively with the Crops Research Division of the Agricultural Research Service and Purdue University to relate coumestrol content of alfalfa to growing area, variety, cutting, harvest year, stage of growth, and disease. It was found that the variation in coumestrol content was essentially nongenetic. In alfalfa samples infected with common leaf spot, a definite increase in concentration of coumestrol was observed as the infection became more severe. The relative amount of the group of phenolics accompanying coumestrol to the amount of coumestrol present was roughly the same in lightly and heavily infected samples. Amounts in disease-free plants were indiscernible. It seems reasonable to assume that the build-up of at least eight other phenolic compounds in plants parallels the build-up of coumestrol during infection.



Mixed feeds for poultry require a yellow pigmentation source. The use of alfalfa in mixed feeds is based principally on its xanthophyll content. The pigmentation potency of various xanthophyll sources was determined by chick feeding experiments. Yellow corn meal, corn gluten meal, pelleted and reground alfalfa meal, lutein, and isolated carotenoid and xanthophyll extracts of alfalfa were compared. The extracts from alfalfa showed higher biological availability of yellow pigment for chicks than did alfalfa, itself, or corn gluten meal. Pure lutein, one of the xanthophylls from alfalfa showed greater pigmentation potency than did the extract which contained a mixture of xanthophylls. The mixed xanthophyll of dehydrated alfalfa meal was as well utilized by hens for egg yolk pigmentation as was that of the alfalfa extract.

2. Alfalfa Processing. A mobile processing unit was operated in Nebraska in cooperation with the State of Nebraska Department of Agriculture and Economic Development, Nebraska Farm Products, Inc., and the Kansas-Nebraska Natural Gas Company. Two cuttings from four plots of alfalfa harvested at 28, 32, 36 and 40-day cycles provided the raw material. Dehydrated alfalfa was separated into three fractions: leaf, stem, and fines. The separate materials will enable the processor to produce feed specifically for the different classes of livestock (e.g., mono-gastric animals and ruminants). Longer intervals between alfalfa cuttings allow a gross increase in yields per acre and strengthen stands. However, usual practice precludes the longer interval because much of the increased yield is in fibrous material as the stand matures. By use of the new leaf separation process, alfalfa meal of 25% to 30% protein grade is still available while yielding as much or more of the stem fraction suitable for feeding ruminants. One dehydrator plant now has several hundred tons of super grade alfalfa at about 27% protein available for market testing, and other operators are keenly interested. Improved control systems are being installed in our mobile processing unit. Tests are to be conducted at Dixon, California in cooperation with the Dixon Dryer Company in 1966. Further tests using commercial-scale equipment will be conducted in Nebraska this summer.

Contract research has been initiated at the University of Nebraska in Lincoln to evaluate, in mixed feeds, alfalfa products that have been processed to lessen the effects of high fiber content. Artificial rumen techniques are being used in preliminary screening studies. Wet ball milling of alfalfa stem increases the rate of cellulose digestibility and the total percentage digested. In one test, cellulose digested in 32 hours increased from 35% to 60% of the alfalfa sample. The State of Nebraska Department of Agriculture and Economic Development and the Nebraska Farm Products, Inc. of Cozad, Nebraska have cooperated in this project.

3. Products from Southeastern Grasses. Contract research is being conducted at the University of Georgia in Athens to study products that can be made by dehydrating Coastal Bermuda grass and pearl millet. Attention has been given to production of higher quality forage and to evaluation and development of

methods for carotenoid pigment analysis. Nineteen cuttings of Coastal Bermuda were made, including 8 cuttings at 3-week intervals, 6 at 4-week intervals, and 5 at 5-week intervals. With 3- to 4-week cutting cycles, good quality was maintained; it decreased slightly as the season progressed to hot weather but increased again as cooler weather started.

With millet, quality can be improved by varying the cutting cycles with the seasons. Millet grown in the Southeast was consistently higher than Coastal Bermuda in moisture content. Both grasses contain high levels of carotene and xanthophyll compared with standard grades of alfalfa meal. Carotenoid stability in dehydrated grasses during storage is similar to that of alfalfa meal and is similarly enhanced by use of the antioxidant ethoxyquin. This research is expected to lead to increases in production of high-quality dehydrated forage products that will be important because of their nearness to the large broiler-producing areas of Georgia and the Delmarva Peninsula. Such products will help regain feed markets lost in these areas because of the high fiber-to-nutrient ratio of alfalfa meal. The products should move into export markets as well.

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## AREA NO. 5. WOOL AND MOHAIR UTILIZATION

Problem. Traditional markets for wool and mohair have been lost to synthetic fibers because consumers prefer garments that hold their pleats and creases, resist shrinkage and wrinkling during washing, and dry quickly. Natural wool and mohair outclass the synthetics in tailorability, comfort in wear, appearance, and hand, but lack certain features now being exploited by the promoters of synthetics. Furthermore, some current processing methods damage, distort, or weaken wool and mohair fibers and injure performance and appearance of the fabric. We need processes that will modify natural fibers to give a range of comfortable and attractive fabrics that resist deterioration in processing and wear. Fabrics must be durably resistant to wear, wrinkling, pilling, abrasion, yellowing, soiling, felting and relaxation shrinkage, acid and alkali weakening, insects, and micro-organisms. New markets in industrial and other uses would develop for new types of fabrics, woven and non-woven, made from natural wools and from blends of wool with modified wools or other fibers. Wool could have a part of the new, rapidly developing market for stretch fabrics if we could practicably impart permanent stretch into wool yarn. Research toward such developments requires fundamental information on the chemical, physical, and structural nature of natural fibers and their modified products.

To sustain a stable sheep and wool industry in the United States, mills must be supplied with processing information on new and improved wool and mohair products. Synthetics have cut into wool markets because the synthetics are uniform in price and quality and because detailed processing information is available from producers.

### USDA AND COOPERATIVE PROGRAM

The Western Utilization Research and Development Division conducts a broad basic and applied research program on wool and mohair to develop new and improved fibers and fabrics that can increase markets. Fundamental research seeks new facts on chemical and physical properties of natural fibers, and we use such knowledge to modify fibers and fabrics so that they will resist degradation by heat, light, chemicals, staining, abrasion, and insects; retain creases; shed wrinkles; and require little care. Department scientists bring research results to the industry through technical publications, public service patents, exhibits, news media and conferences.

The Federal program is conducted at the Division headquarters at Albany, California; by contract in Durham, North Carolina, and Washington D.C.; and by grant funds under P.L. 480 in India, West Germany, Sweden, England, and Finland.

The Federal program of research in this area totals 24.8 scientist man-years, including contract research equivalent to approximately 1.5 scientist man-years per year. Of this number 9.7 are assigned to chemical composition, physical properties and structure; 7.9 to chemical and physical investigations to improve products; and 7.2 to technology--process and product developments. In addition, the Division sponsors six research grants under Public Law 480.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 5 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition, Physical Properties and Structure

1. Measurement of Physical Properties. Research aimed at improving the properties of wool has relied heavily upon fundamental knowledge of structure, chemical properties and physical behavior. In studies of this nature, it was found that measurement of stress relaxation in wool fibers can be used to determine diffusion of water and certain other chemicals into wool. The measurement of diffusion coefficients until now has been difficult, but the development of this new stress-relaxation method greatly simplifies the procedure. It will have important applications in dyeing.

For the evaluation of fabric surface characteristics a new photographic method was developed. The method has been applied in making critical measurements of the surface fuzziness of treated and untreated fabrics under various conditions of wear.

Further research was carried out on methods of testing of physical properties of bundles of wool fibers and on correlation of physical measurements of abrasion resistance with actual wear tests. The correlation of results of accelerated tests with actual wear trials of fabrics is of importance for the speedy and practical development of chemically modified wool and mohair fabrics.

Problems related to crimp in wool fibers are being subjected to critical analysis under a P.L. 480 grant recently executed in Israel.

Physical measurements involving particularly ultrasonic absorptions are being applied to a study of molecular processes in model substances related to wool. This work is being carried out under a P.L. 480 grant to the University of Allahabad in India. Basic information on the internal structure of fine, coarse, and chemically modified wools is being developed through the application of low-angle X-ray diffraction techniques. This difficult research, which is being performed under a P.L. 480 grant at the German Wool Research Institute at Aachen, is providing insight into the structure of wool keratin and should lead to information concerning the



amino acid distribution along the fiber axis. Further understanding of the molecular structure of wool is being obtained through a P.L. 480 grant to the Karolinska Institutet in Stockholm, Sweden. Cross-sections of different levels of developing fiber cortex have been studied with electron microscopy which showed the location of formation of fibrils and filaments and presented evidence of the aggregation of the filaments into twisted cables.

2. Study of Chemical Composition. The physical separation of wool fiber into protein fractions has been followed by chemical analysis of the components. Significant differences were found in the amino acid composition of the protein fractions. These studies are aimed at increasing our understanding of the wool protein structure and its relationship to properties observed in processing and use.

A study of the chemical structure of wool protein in the neighborhood of the cystine residues is being carried out at the Wool Industries Research Association in Leeds, England, under a P.L. 480 grant. The way the amino acid units are arranged in the protein in the vicinity of the cystine residues must be the most important reason why some of the cystines are more reactive than others. Properly manipulated, the cystine residues confer upon wool fabric desirable properties such as set and crease resistance; on the other hand cystine may also be involved in yellowing and weakening of wool under adverse conditions. Following the lead provided by earlier work in this Division, the grantee has applied acrylonitrile as a stabilizing reagent for thiol groups in reduced wool protein, a technique which facilitated fractionation of the protein after enzymic digestion. Acrylonitrile also functioned as a blocking agent for terminal amino groups of peptides, an application potentially useful in the analysis of the amino acid sequences in wool protein.

3. Study of Effects of Light. Information on the effects of light upon wool is providing further insight into color changes in wool after exposure to strong light. This research is establishing a foundation for the development of treatments to eliminate undesirable effects and, in addition, has provided a lead worthy of exploration for a photobleaching process.

#### B. Chemical and Physical Investigations to Improve Products

1. Improvement in Dimensional Stability of Wool Fabrics. Exploratory studies on treatments to increase wool's dimensional stability were continued. It was found that in the presence of suitable catalysts (e.g., ethylene carbonate), toluylene diisocyanate reacts with wool to form a protective surface coating. Another useful combination is sebacoyl chloride, which is one of the WURLAN components, and ethylene imine, a material which is becoming commercially available at an attractive price.

Extensive tests were made with polymeric finishes for wool with a treatment similar in some respects to WURLAN. The new treatment is tentatively being

identified as WURLAN-II. In this new scheme, wool is treated with reactive polymers, and these polymers are subsequently crosslinked by a second chemical treatment. When crosslinked, the polymeric finishes are insoluble and are durable through repeated launderings. Many suitable polymers are commercially available, and with this new technique they greatly widen the range of protective finishes that can be applied to wool, including several new types of finish which were unattainable with the original WURLAN treatment.

2. Improvement in Water- and Oil-resistance of Wool Products. Several new classes of polymers containing fluorine were synthesized and their properties studied. Starting with a new and relatively cheap fluorochemical, hexafluoroacetone, novel polymers were prepared which show extraordinary promise as protective finishes for textiles. These products should be considerably cheaper than any fluorochemical finishes now commercially available. Publications describing these compounds have already generated great interest in the industry.

3. Improvement in Luster in Wool Fabrics. Luster is a quality frequently associated with luxurious fabrics and durable luster in certain types of wool fabrics is highly desired. Previously, chemical and mechanical treatments produced, at best, a temporary luster in wool. Research performed at the Harris Research Laboratories, Washington, D.C., under a research contract, has endeavored to delineate the most important factors influencing this rather evasive quality and to find treatments to enhance it. Chlorination or other treatments which chemically affect the surface of the wool fibers tend to increase the luster but at the same time to damage the strength and abrasion resistance. It was found, however, that certain fluorochemical polymers increase the luster without damage to the wool. The beneficial effect is not as large as desired, and so efforts are being made to combine mild oxidative surface treatment with the best of the polymeric finishes.

4. Exploratory Chemical Investigations. A study of wool's adsorption of selected ions was begun under a P.L. 480 grant to the Ahmedabad Textile Industries Research Association in India. This research is aimed at providing new directions for improvements in chemical modification, processing and use. Experimental results discussed in the first report are too brief for a fair appraisal of the project.

A P.L. 480 grant to the Textile Research Association of Helsinki, Finland on the interrelationships of finishing treatments, moisture, fabric handle, and tailoring qualities was concluded. Among the conclusion are the following: (a) the stability of surface smoothness after mechanical washing is better for top-dyed fabrics than for piece-dyed; (b) top-dyed fabrics are also superior to piece-dyed in creasing properties and are more stable dimensionally; (c) on top-dyed fabrics, setting treatments affect the fabric properties far more than does the mechanical action of different scouring and milling treatments; (d) from the point of view of garment manufacture, flat-setting treatments make it more difficult to produce a good seam.

One of the most important results of this project was the development of a new method for estimating the creasing properties of fabrics. The method can easily be adjusted to correspond with the conditions in which the garments are most likely to be used, e.g., in cold or heat, dry or moist climates, etc.

5. Improvements in Properties Through Application of High Energy Radiation. Research in this area, largely of a fundamental nature, is being conducted under contract at the Camille Dreyfus Laboratory of the Research Triangle Institute, Durham, N.C. It has been found that through the application of high energy radiation vinyl monomers can be polymerized and grafted to wool. When the amount of grafted polymer is less than about 20% by weight, the wool is only very slightly changed in gross physical properties, such as stiffness or texture. However, refinement of physical measurements will be needed to evaluate the changes in certain other essential properties. This work holds considerable promise, and the publications on the early phases of this project have already attracted wide interest.

#### C. Technology--Process and Product Development

1. Washable Wool Fabrics; WURLAN and WURLAN-II. Research and development on the application of the WURLAN treatment to wool top showed that a high degree of shrink-resistance with minimum effect on processing characteristics or softness of hand can be achieved. Key factors were defined as proper selection of reactants, optimization of treating conditions, and proper design of equipment. The addition of non-ionic softeners and fiber lubricants was found to be helpful. The WURLAN treatment, now in growing commercial use, is the first feasible application of a resin finish to wool top to achieve shrink-resistance.

Extensive pilot plant studies on the crosslinking of reactive polymers (see also 5-B-1) provided information to be used in later mill trials. Excellent stabilization of wool was achieved in pilot studies when wool was treated with reactive isocyanate polymers, which were then crosslinked with polyamines. Chemical costs of treatment should be less than for the original WURLAN, because the reactive polymers are commercially available at about half the cost of WURLAN chemicals. Much more work will be required, of course, in pilot plant and mills in order to determine optimum treating conditions for possible commercial applications.

2. Construction of Fabrics. Studies have been made of the variables in yarn and fabric construction, woven and knit, comparing not only physical construction factors such as yarn twist, but also comparing WURLAN-treated and control yarns. Efforts are being made to determine the critical factors affecting the efficiency of knitting of these yarns and the performance characteristics of the fabrics made from them.

To better understand the underlying physics and mechanics of the knitting process, a mathematical analysis has been made of the forces occurring in the weft-knitting process on conventional machinery. This study showed



that present conventional machine designs are inefficient. Large forces are created in the knitting zone, causing the yarn to break out, and knitting speeds are limited because of poor engineering. Alternate designs to increase wool knitting speeds and efficiencies have been proposed and will be evaluated.

The basic variables governing wear-wrinkling performance of light-weight wool fabrics were investigated in a contract project at the Harris Research Laboratories, Washington, D.C. The eighteen special fabrics designed, woven and finished for study included ten worsted, six woolen, and two wool/mohair blends. A series of chemical treatments was also made on portions of these fabrics. A total of 66 experimental fabrics varying in construction and chemical treatment were studied for wear-wrinkling behavior. All of the fabrics were subjected to standard laboratory tests, and from 12 of the fabrics representing the most important variables in construction and treatment, men's slacks were made and worn in two service tests. Correlations were made between the laboratory and service tests.

On the basis of this study, a wool fabric designed for minimum wrinkling in service should have the following features: it should be woven in a relatively open weave; it should be as thick (heavy) as consistent with its intended use; and it should be made from fibers as coarse as possible consistent with comfort, drape and ability to spin to the desired yarn size. Setting with bisulfite although it did not affect crease recovery, improved the overall garment appearance. WURLAN treatment had no effect on wear-wrinkling behavior.

The information obtained will be useful as a basis for further studies, by providing a background on the interrelationships among various constructional factors and chemical treatments with respect to the wear-wrinkling properties of worsted and woolen fabrics.

3. Wool-Cotton Blend Studies. Work in this area was initiated with a study of available wool/cotton fabrics and has progressed to the point of design of new blends for further testing. The WURLAN treatment alone imparts excellent shrink-resistance to wool-cotton blend fabrics. In combination with new delayed-curing treatments now in use on cellulose, outstanding durable-press qualities can be achieved. Experimental durable-press wool-cotton shirts, WURLANized and processed by the Koratron type of delayed cure, have gone through 20 home-laundrying, tumble-drying and wearing cycles and have retained their press without the need for any ironing.

Although these preliminary results are highly promising for the use of wool in modern durable-press blends, many problems remain to be solved. Fabrics thus treated tend to lose an appreciable degree of abrasion resistance and tear strength. There is also need for a delayed-curing resin that will crosslink with the cellulosic component at lower temperatures than now conventionally used for such curing.

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## AREA NO. 6. CITRUS AND SUBTROPICAL FRUIT UTILIZATION - FOOD

Problem. The economic stability of the citrus and subtropical fruit industries in the Western Region is dependent upon effective utilization of fruit that cannot be accommodated on the fresh fruit market. The utilization of surplus or wholesome but blemished fruit provides the margin necessary to assure adequate returns to the farmer and continued development of stable markets. Ineffective utilization of products and continuously increasing processing costs are resulting in decreased returns to the growers. The California-Arizona grapefruit industry is encountering difficulty in disposing of both fresh fruit and processed grapefruit products. The pineapple and subtropical fruit industry in Hawaii must find practical methods for processing its products for export in order to prevent the accumulation of burdensome surpluses. The navel orange industry in California is hampered by the unavailability of satisfactory processes for the utilization of navel oranges. Juice extracted from early fruit, and during some seasons from almost all of the navel oranges, contains substances that impart an intolerable bitter flavor to juice products after mild heat-processing or after standing at ambient temperature for a short time. Large new plantings of navel oranges may be expected to aggravate the utilization problem. Deterioration of the flavor and color of these and other processed citrus and subtropical fruit products imposes severe limitations upon the economic stability of the industry.

Information is needed on the chemical composition of citrus and subtropical fruits and their products and byproducts as a basis for the development or application of new and improved methods of processing and for the production of new and improved food and industrial products and pharmaceuticals. Special attention needs to be given to the nature of the chemical changes involved during pre-treatment, processing and handling which lead to the formation of off-flavors, -colors, and -odors in processed products.

### USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a concentrated program of fundamental research on citrus and subtropical fruit and its application to industry problems is conducted at the Division headquarters at Albany, California; at the Fruit and Vegetable Chemistry Laboratory in Pasadena, California; at the University of Hawaii, Honolulu; by contracts at Riverside and South Pasadena, California, and Tucson, Arizona; and, under a P.L. 480 grant, in Bogota, Colombia. Investigations are conducted on the composition of citrus essential oils, the flavonoid compounds and other citrus constituents that are related to off-flavors and darkening of citrus products, the natural flavor components of oranges, the enzyme systems that are involved in the appearance or disappearance of constituents and structures of plant tissues, and the constituents of dates that affect the quality and stability of products. The findings of such

research are applied in the development of new and improved citrus, tropical, and subtropical fruit products.

The Federal program of research in this area totals 19.0 scientist man-years, including contract research equivalent to about 1.6 scientist man-years per year and two scientists whose salary is provided under Memorandum of Understanding by the Lemon Products Technical Committee. Of the total, 7.4 are assigned to investigations on flavor; 5.2 on color, texture and other quality characteristics; 0.5 on microbiology and toxicology; and 5.9 on technology--process and product development. The Division supervises one research project supported by a P.L. 480 grant.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 19 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Flavor

1. Components of Aroma. Alpha- and beta-sinensal, which are unstable isomeric sesquiterpene aldehydes, were isolated from orange oil and shown to be important to natural orange aroma. These compounds have remarkably low odor thresholds (about 0.05 parts per billion in water)--very few compounds can be detected at such dilute concentrations. Nevertheless, they are the first compounds to be isolated from orange oil that impart a unique orange-like aroma; they obviously are important in the overall profile of orange flavor.

Gas chromatographic procedures have been further refined so that now more than 50 principal components of cold-pressed lemon oil can be analyzed without prior separation of the components into classes of compounds. Qualitative and quantitative evaluation of a great number of components can be made by feeding the effluent from a temperature-programmed capillary gas chromatograph directly into the ionization chamber of a time-of-flight mass spectrometer. These more rapid methods for analyzing whole citrus oils make it possible to establish standards to improve the quality of these products in commerce.

Nootkatone was identified as an important flavor component of grapefruit and its gross structure established as reported last year. Recently the stereochemistry of the molecule was investigated, and the stereochemical assignments given previously to nootkatone and its derivatives were confirmed. The orientation of the three-carbon side chain was determined to be equatorial, which completes the structure, relative stereochemistry, and absolute configuration of the molecule. This establishment of the complete constitution of nootkatone is essential to evaluation of its role in the flavor of grapefruit products.

Investigation of ways to stabilize flavor in tropical fruit concentrates is supported by a P.L. 480 grant to the Institute of Technological



Investigations in Bogota, Colombia. Volatile constituents of guava and curuba are being identified. Extracts of these fruits were analyzed for free acids by studying the methyl esters of the free acids with gas chromatography. Commercially produced tropical fruit products were chromatographically analyzed to measure freedom from contaminants. Several compounds, mostly esters, were synthesized for use as standards in evaluating tropical fruits. Tentative identity has been established for 40 components of guava and 18 of curuba. Recovery and concentration of guava volatiles has given products with aromas that closely approach the aroma of the fresh fruit. Concentrated recovered volatiles of guava and of curuba have been incorporated into several inert carriers (i.e., sugars, sugar mixtures, and sugar derivatives) and are now undergoing storage tests at several temperatures.

2. Citrus Bitterness. Basic research on the flavonoid constituents of citrus products has revealed much useful information about these components that are related to bitterness. Another type of compound, limonoid, is also involved in citrus bitterness. Three limonoids exist in significant quantities in navel orange juice, but only one of them, limonin, causes bitterness. Limonin has now been found, both in fresh grapefruit juice and in grapefruit juice products. This intensely bitter triterpenoid occurs in grapefruit juice samples at levels above its taste threshold. Past experiments with flavonoid debittering processes, based on treatment of grapefruit juice with naringin-hydrolyzing enzymes, were difficult to interpret because limonin had not been discovered in the juice, and the enzymes removed only the bitterness caused by naringin. The new discovery should lead to grapefruit juice that is less bitter, if we can develop appropriate new processes to remove limonin, or if new varieties can be bred in which limonin content is either very low or absent.

In contract research conducted by the Stanford Research Institute in South Pasadena, California, a satisfactory analytical method was developed for quantitative determination of limonin in navel orange juice. The procedure is sensitive, rapid and simple. When it was tested on several samples of orange juice of known limonin content, good recovery and low blanks were obtained. In orange juice prepared with known, very small amounts of limonin, recovery of limonin was 90% or better. The error we believe was largely due to mechanical loss of limonin during the extraction step. Interfering substances are negligible with this method of analysis. The new method should make it possible to determine routinely the limonin content of navel orange juice or to analyze oranges to determine whether or not their limonin content is so high that the juice made from them would not be salable. The bitterness of navel orange juice has long been known to be partly determined by the root stock on which the oranges were grown. Now, knowledge of the limonin contribution from various root stocks will be valuable in breeding new varieties and could lead to the development of a new non-bitter navel orange. The new method may be even more important for grapefruit juice. However, there are substances present in grapefruit which interfere with the analytical method to a much higher degree than is the case with navel orange juice.

Experiments were started to determine the metabolic paths that lead to the biogenesis of limonin. It is possible that ichangin may be a less bitter intermediate in the formation of limonin. Rutaevin, an oxidation product of limonin that is slightly bitter but not nearly as bitter as limonin, was isolated and its structure determined. A knowledge of the sequence of compounds that lead to the formation of limonin will aid in devising a method to control bitterness.

In grapefruit, naringin accumulates during the first few months of fruit development. After naringin ceases to accumulate, the fruit undergoes a three- to four-fold increase in dry solids. Our studies have shown that profound changes also occur in the pattern of polyphenols at the time naringin accumulation ceases. Thus, a biological mechanism seems to regulate naringin accumulation. This may be a further clue to eventual control of citrus bitterness.

## B. Color, Texture, and Other Quality Factors

1. Citrus Carotenoids. We are investigating the chemical changes that occur in the yellow and orange carotenoid pigments of citrus fruit and its products. The major carotenoid constituents of desert grapefruit, lemons, and Valencia oranges are being isolated and characterized. In green fruit, carotenoids are subject to a degradation that is probably enzymic; however, there is a net synthesis of total carotenoids in both the endocarp and the peel of immature desert grapefruit. As the fruit ripens and the carotenoids decrease, esterified xanthophylls, which also are yellow pigments, increase. The ripe fruit, on disappearance of the green chlorophyll pigment, increases in carotenoids of the endocarp, while the peel shows a decrease in total carotenoid content. Changes in carotenoid pigments in the endocarp and peel of desert grapefruit show great variability during senescence and storage, that is, whether the grapefruit remains on the tree or not. We hope to trace the degradation of the complex carotenoid mixture of desert grapefruit to help us develop better methods for preserving color in grapefruit products.

2. Composition of Dates. We now know that the brown coloration of dates is the result of at least three different chemical pathways: (1) non-enzymic, oxidative browning of the insoluble leucocyanidin tannins; (2) enzyme catalyzed oxidative browning of the dactylifric acids, epicatechin and other water-soluble flavans; and (3) nonenzymic, nonoxidative, reducing sugar browning. In addition, when dates are heated higher than 140-160° F., an undesirable reddish discoloration develops. The red pigment may be caused by high-temperature conversion of leucocyanidin tannins. This knowledge that dates darken and discolor by a number of chemically distinct pathways is of considerable importance both scientifically and commercially. Processing conditions for dates can now be chosen to minimize darkening and discoloration of the fruit, and the information should be useful in the development of new processes and products. There is a very good chance that darkening of other dried fruits takes place by chemical pathways similar to those found in dates. Research on dates has been largely supported by the Date Administrative Committee of Indio, California. Financial support has been terminated, and research on dates is being continued under contract with the University of California at Riverside.

### C. Microbiology and Toxicology

1. Pharmacology of Dihydrochalcones. The new methods for converting the bitter citrus flavonoids, naringin and neohesperidin, to intensely sweet dihydrochalcones may lead to commercial use of the new compounds as low-calorie food sweeteners. Toxicity levels of the dihydrochalcones have been determined by rat-feeding tests, and the promising results have been reported to interested industrial concerns. Two of the companies that hope some day to market these products have volunteered to prepare quantities of naringin and neohesperidin dihydrochalcones for further testing by the Pharmacology Laboratory of the Western Utilization Research and Development Division.

### D. Technology--Process and Product Development

1. Citrus Products. Chemical methods are being developed to characterize lemon juice and lemon oil, and these methods are gradually being adopted by the industry and regulatory agencies for quality control and for determining authenticity of products. Rather large fluctuations were observed in the total amount of phenolic compounds of lemon juice--studies are underway to determine the cause. Chromatographic procedures were developed for quantitative estimation of individual polyphenolic compounds in hydrolyzed lemon juice. Since the total phenolic measurement is important in characterizing lemon juice it is necessary to know the individual components. Studies have been published on analytical methods for determining the total amino acid and total polyphenolics contents of lemon juice, and on the effects of fruit storage and processing variables on lemon juice composition.

The development and testing of new products from desert grapefruit are underway in contract research at the University of Arizona in Tucson. Combinations of grapefruit juice, guava juice, and grape juice appeared promising in preliminary studies, and samples have been stored for long-term stability testing. Taste panels indicate that diced grapefruit can be an acceptable substitute for sectioned grapefruit in salads. Such a substitution would materially reduce the cost of canned, frozen, and chilled grapefruit products. Studies are underway to enzymatically tenderize and debitter desert grapefruit to improve acceptability. Formulations are being tested to use grapefruit juice as an acidulant in salad dressing, canned nectars, and baby foods; as a browning control agent for french fried potatoes; and as an agent to inhibit enzymic browning of peeled fruit. When grapefruit juice was added to canning syrups, it imparted an improved fresh fruit flavor to canned apricots.

Foam-mat drying of fruit juices on a large scale is being studied at the Southern Utilization Research and Development Division at Winter Haven, Florida, with cooperation and support from the citrus industry in that state. Large runs of grapefruit juice have been made for use in market development tests. In-house investigations on foam-mat drying at the Western Division have been confined to demonstration runs to test the feasibility of dehydrating various fruit juices and other liquid foods.



2. Subtropical and Tropical Fruit Products. At our Hawaii field station, subtropical fruits are being concentrated and dehydrated to determine the processing quality of locally grown varieties of bananas, guavas, pineapple, papaya, and passion fruit, and to demonstrate processing methods that may aid in expanding local food industry. Two varieties of bananas were drum-dried, air-dried, or freeze-dried and then tested for storage stability. Analyses after one year storage have been completed. Storage temperature was an over-riding factor in rate of deterioration, although significant differences due to moisture content were observed. Low-moisture products (less than 4%) were more stable than high-moisture products (17-18%). Sulfur dioxide enhances the quality and stability of dried bananas. Mangoes were drum-dried to produce a good product, but other fruits appear to require special pre-drying preparation if drum-drying is to be feasible. Freeze-drying produced satisfactory products from all of the fruits tested. Quality of guava purée was improved by deaeration prior to freezing; deaeration also increased bulk density. Six months' storage at zero caused some color loss if the purée had not been deaerated. On thawing, the deaerated purée had less tendency to separate into pulp and a clear liquid than did the untreated sample. The separation of skins and seeds from papaya purée has been a major hurdle to developing a commercial product. We are working on a scraping devise that can remove the bitter skin from the flesh, and also on using standard pulper and finisher equipment to remove seeds from the flesh. Diversified fruit products will help stabilize the price of these fruits by reducing their perishability and making them available for shipment to markets away from Hawaii.



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## AREA NO. 7. DECIDUOUS FRUIT AND TREE NUT UTILIZATION - FOOD

Problem. Fruits and nuts are valued for their unique flavor, color, and mineral and vitamin content. In the period of abundance at harvest time, markets are glutted and growers often do not get an adequate return. Crops are perishable, and processing to preserve their unique qualities is difficult. No processed fruit retains completely the fresh values, although many highly acceptable products exist and about half of the fruits and nuts marketed in the United States are processed. Processing makes these commodities available to consumers the year around, and has opened new markets for producers. The proportion of processed commodities is steadily increasing but is dependent upon a continuing flow of new knowledge. Processing to preserve color, flavor, texture, and nutrients presents many problems, and each new product requires the application of much scientific and technological skill.

The continued supply of preserved fruits is largely dependent upon relatively inexpensive sources of raw material. Decreasing supply and increasing cost of harvest labor is leading rapidly to the development of mechanical harvesting methods. Mechanical harvesting does not allow the sorting and careful handling of traditional hand picking. The processor must deal with trash, and bruised, cracked, immature, and over-ripe fruit along with prime quality. Research is necessary to develop new processes and products to reduce costs and utilize such raw material in the processing plant.

The freezing process for preserving certain fruits keeps products at near fresh fruit condition, but many problems remain unsolved. The enzymatic browning of frozen peaches and the sloppy texture of frozen strawberries on thawing are two examples.

Frozen fruits require expensive low-temperature storage and transportation facilities. This expense can be greatly reduced by removing a portion of the water from the products. Orange and other fruit juice concentrates are well established in U.S. markets, and dehydrofrozen apple slices (rapid drying to 50% bulk weight and then freezing) are just becoming well established. Many other fruits and fruit juices should be amenable to concentration. Any frozen product, however, is not as well adapted for export as those which do not require refrigeration.

Maximum weight reduction, as well as less restrictive storage requirements, can be achieved through dehydration. The drying of fruit juices has been successfully accomplished by the vacuum puff drying and foam-mat drying processes. New methods are being developed to dehydrate pieces of fruit with excellent retention of color and flavor. Extension of laboratory procedures to pilot- and commercial-scale operations must still be done. Flavor recovery and the incorporation of recovered flavor in solid carriers

for addition to the dried products require technological and basic chemical study. Aroma recovery techniques developed for fruit juice concentrates are being improved but require more work. Dried fruits are now widely used in the U.S. and abroad. Their popularity would grow if stable, higher moisture dried fruits were available and if lower levels of sulfur dioxide could be used without loss of quality.

Container costs for canned fruits limit the shipment of these products overseas. A solution of the container problem may be found in the use of lightweight fiber, foil, or plastic containers and aseptic filling procedures.

Fruit growers need new varieties of tree fruits and berries suited to processing and resistant to diseases endemic to each region of production. Utilization research is required in cooperation with farm research to assure growers of a market for fruit in the processing industry.

#### USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic and applied research on deciduous fruits and tree nuts is conducted at the Division headquarters at Albany, California; in field stations at Pasadena, California, and Puyallup, Washington; by contracts in Davis and Los Angeles, California, Fort Collins, Colorado, Geneva, New York, and Chicago, Illinois; and by grant funds under P.L. 480 in Israel, Taiwan and India. Fundamental research is conducted on fruit constituents associated with flavor, color, and texture of fruit products. The work includes development of laboratory tools to isolate and characterize components, investigation of such components as they occur naturally and as they are altered by operations involved in preservation, and study of the relationships between the components and the product values being preserved. Applied research is conducted to develop new and improved processes and products that will increase utilization of fruits and tree nuts, including the development of high-quality concentrated and dehydrated products and more stable shelled tree nuts and the selection of improved processing varieties. Pioneering research on plant enzymes is also conducted.

The Federal program of research in this area totals 35.7 scientist man-years, including two scientists whose salaries are provided by two co-operators (Dried Fruit Research Advisory Committee, whose membership represents the California Raisin Advisory Board, the Dried Fig Advisory Board, the California Prune Advisory Board, and the Dried Fruit Association of California; and the Walnut Control Board - one each) under Memoranda of Understanding; and six grants and contracts providing research at a rate of approximately 3.9 scientist man-years per year. Of the total number, 0.6 are assigned to investigations on chemical composition and physical properties; 7.7 on flavor; 13.3 on color, texture and other quality characteristics; 3.3 on microbiology and toxicology; and 10.8 on technology--process and product development. In addition, the Division sponsors basic research on fruit by means of four P.L. 480 grants.

## PROGRAM OF STATE EXPERIMENT STATIONS

A total of 50 scientist man-years is devoted to this area of research.

### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

#### A. Chemical Composition and Physical Properties

1. Walnut Fatty Acids. Precise measurement of fatty acids in oils obtained from fresh walnuts and from kernels that had been subjected to accelerated storage revealed no difference in these components although the stored samples were damaged severely from an organoleptic standpoint. If, as has been generally assumed, oxidation of unsaturated fatty acids in lipids is responsible for rancidity of stored walnut kernels, the oxidation must be of only minute amounts of any of the major fatty acids. Or perhaps some unknown minor constituents are intimately involved in rancidification. High-resolution micro-preparative gas chromatography techniques have been developed to isolate highly purified compounds in microgram quantities. The isolated material can be carried to other locations for micro-analyses such as mass spectrometry and infrared spectrometry. These microtechniques permit a fast comprehensive study of chemical changes in major and minor lipid constituents of walnut oil and an evaluation of their roles in the development of rancidity.

2. Alleged Allergenicity of Chlorogenic Acid. Chlorogenic acid is a common component of fruits and most other plant materials. Research elsewhere on the allergy of green coffee beans led to a series of publications that implicated chlorogenic acid as the allergenic substance giving intense immediate skin reactions. Using allergic serum transfer tests in non-human primates and cooperating in tests with human volunteers, we have rigorously tested this hypothesis. We did not find chlorogenic acid to be an allergen in human atopic hypersensitivity. However, antigenic properties were observed in the protein-containing fraction of green coffee beans. Chlorogenic acid isolated from fruit elicited no allergic reaction, either.

#### B. Flavor

1. Methods and Equipment. Collection of purified samples from gas chromatograph effluents has been made more efficient and simple by using Teflon tubes for 1-mg. quantities and stainless-steel wool in glass tubes for 100-mg. quantities. Relative retention times with various stationary liquids have been studied to determine quantitatively the interaction between solute and solvent. With the information obtained, we now can choose logically a satisfactory stationary liquid for separation of specific mixtures, to make more efficient columns, and provide better resolution.

Large numbers of esters are found in fruit volatiles. Isomers are difficult to separate, so spectroscopic data and organoleptic descriptions that would be helpful in providing a better understanding of fruit aroma are meager.



To attack this problem, we obtained the 8 pentyl acetate isomers and cyclopentyl acetate from commercial sources or by synthesis, and rigorously purified them by preparative gas chromatography. Then we determined the purity of each sample by high resolution analytical gas chromatography, and obtained and recorded the infrared proton magnetic resonance and mass spectra of each. Taste panels evaluated each sample for threshold and made discrimination determinations. Odor thresholds ranged from two parts per billion parts water for 2-pentyl acetate and 3-methyl-1-butyl acetate to 30 parts per billion for 1,1-dimethyl-1-propyl acetate. Although most of the compounds were given the general qualitative odor description of fruity-floral, the panel was reliable in distinguishing a difference between the individual isomers.

2. Fruit Volatiles. Three varieties of grapes (Concord, Muscat of Alexandria, and Grenache) have been analyzed by gas liquid chromatography-mass spectrometry. Of the many volatile components from Concord grapes, 16 have been identified. Ethyl acetate and ethanol were found in greatest quantity; the next most abundant component was 2-methyl-3-butene-2-ol. Other components are being identified. Some 60 components have been identified in the volatile constituents of the juice of Muscat of Alexandria grapes. Several components, including propyl benzene, 3-methyl-biphenyl and diphenyl ether, are unique to this fruit or have been overlooked in similar research with other fruits. The flavor and aroma of the muscat grape is considered unique, and these unusual volatile components may be a reflection of this distinct characteristic. Similar methods have been used to investigate the fruit volatiles of Grenache grapes. Hexanal was by far the largest constituent of the oil obtained when a 100-fold essence from free-run juice was extracted with isopentane and partitioned between propylene glycol and isopentene. Large amounts of isomers of amyl alcohols and other alcohols were obtained in addition to a large number of aldehydes, ketones and esters.

Organic volatiles were extracted from 150-fold red Delicious apple essence, and fractions separated by gas liquid chromatography. Taste panels sniffed a portion of the column effluent to identify fractions with characteristic apple and apple-like aroma. Relative odor intensities of these fractions were determined by threshold concentration measurements. Of four important fractions, the one with the most intense odor was present in the lowest concentration. Apple essences which had been held for one year at different temperatures lost significant amounts of certain components as measured by area under gas chromatograph peaks.

Research aimed at developing better and more uniform flavor in canned ripe olives is being conducted by contract with the National Cannery Association's Western Laboratory in Berkeley, California. Olive samples designated by industry spokesmen as having certain typical off-odors were assembled and evaluated at eight processing plants. Industry tasters gave significant off-flavor assignments to only one sample which contained an excess of ferrous glucanate. Other samples judged as off-flavor by some were not so designated by others. Analyses of these samples were made for six compositional factors: volatile fatty acids, squalene, polyphenols, total tannins, iron, sodium, and



volatile carbonyl compounds. Olive samples judged good in flavor had methyl ketone content of less than 2 parts per million. Olive samples described as having bitter, storage, or Zapatera off-flavor had methyl ketone content of 2.5 to 17 parts per million. Experimental packs of Manzanillo olives were made and processed after different storage periods in brine. Only four samples that had been in the brine for 15 and 20 weeks were distinguished by a trained laboratory taste panel as being different from the other samples. No difference was found among four samples which differed in final pH over a range of 9.05 to 7.13. Volatile carbonyl content increased rapidly in the brine during the early part of storage and then decreased as storage time lengthened. In experimental packs of olives, no significant correlation of product flavor with volatile methyl ketones was observed, although such a finding appeared in several lots of commercially packed olives.

### C. Color, Texture and Other Quality Factors

1. Fruit Pigments. Cooperative studies conducted with the Crops Research Division have demonstrated that color hue and shade, and also stability of anthocyanin pigments, are dependent upon four factors: (1) a particular anthocyanin pigment; (2) a complexing metal salt, such as aluminum or ferric ion; (3) a phenolic co-pigment such as chlorogenic acid, quercetin, or catechin; and (4) an acid that will not compete with a metal ion in chelation with the co-pigment. It has long been known that the colors of many red and blue fruits and flowers are due to anthocyanin pigments. A typical pigment would be red in acid and blue or purple in a more basic solution. However, it has not been possible to explain the blue color at physiological pH of fruits and vegetables, nor to account for the stability and intensities of the colors at the natural pH levels. The pigmentation of hydrangea flowers may be used as an example of anthocyanin reactions. Both red and blue hydrangea blossoms contain the same anthocyanin pigment and undoubtedly the same co-pigment. In the blue flowers, an insoluble complex accounts for the color. In the red flowers, either insufficient metal is present or it is unable to chelate with the co-pigment because of the competitive action of citric or tartaric acid apparently not present in the blue flower. Similar mechanisms probably influence color and color stability of purple and red grapes, plums, and berries.

Investigation of the natural tannins and leucoanthocyanins in fruits is being conducted in contract research at the University of California at Los Angeles. Dimeric proanthocyanins of the avocado are composed of two flavan monomers containing 15 carbon atoms each. The reaction mechanism of this dimerization is being investigated by means of a model system involving methylation and reduction of dihydroquercetin.

The phenolic components of apple juice that complex with proteins and settle out of clarified juice are being investigated in contract research at Colorado State University. Extensive analyses of apple juice and sediments obtained by accelerated storage were made. The sediments were demonstrated to be a complex of protein and oxidized polyphenolic compounds. The loss of

phenolic constituents from the juice during accelerated storage could be followed by thin-layer chromatographic analyses. Sulfite and ascorbic acid added as antioxidants during milling and pressing of apples did not prevent sedimentation of clarified juice. Mineral analysis of the sediments showed the presence of calcium, magnesium, copper, iron, potassium and sodium, indicating a heterogeneous nature of the sediment. Leucoanthocyanidins and catechins were found to be the principal precursors of the phenolic components of the sediment. Chlorogenic acid did not appear to be involved.

The phenolic substrates of enzymic browning of fruits and the methyl-transferring enzymes that alter the substrates to prevent them from turning brown are under continuing investigation. To obtain information on stability and specificity, we have used exchange resins and dextrin gels to purify plant catechol O-methyltransferase. With carbon-14 as a tracer, we are determining methyl transfer in biosynthesis of methylated constituents of plants. Control of native methyl transferase in apples by temporary alteration of surface pH allows phenolic browning substrates to methylate so that apple slices can be preserved for two to three weeks under refrigeration. With this means of color control, apple slices remain crisp. If color is protected only by sulfur dioxide, two adverse side effects are the presence of a sulfite flavor and a loss of cell turgor and crispness. A commercial processor in Wisconsin has followed our suggestions and is applying our process to the preservation of peeled and sliced apples and cored apples for the restaurant and bakery trade.

Research at the University of Delhi in India is supported by P.L. 480 funds to determine the role of leucoanthocyanins in the development of natural pigments and the darkening of deciduous fruits during processing and storage. Two anthocyanins were extracted from plum peels and shown to be derivatives of pelargonidin. No other polyphenols were obtained from the methanolic hydrochloric acid extracts. Similar extracts from peels of three varieties of red apples were found to contain pelargonidin and two flavonoids. Two leucopolymers were obtained from grapes; one turned blue in the presence of ferric ion, the other remained unchanged. The one with the blue reaction was shown to be a derivative of leucopelargonidin. The seeds of the grapes also were found to contain leucopelargonidin.

P.L. 480 funds support an investigation of enzymatic browning in deciduous fruits at the Hebrew University in Jerusalem. The activity of phenolic oxidase enzyme activity was followed in developing apples from fruit set to harvest time. O-diphenyls had peak concentration early in the fruit development; at maturity the concentration drops, apparently because they are converted to other compounds or synthesis stops. Catechol oxidase activity shows a peak after that occurring for the enzyme of O-diphenyls. As the fruit ripens, activity drops sharply, and in the ripe apple only a slight amount of soluble enzyme is found. Findings were correlated with the rate of browning of apple slices and the location of enzymes and their substrates in fruit. A number of apple varieties were compared for keeping quality, O-diphenyl content, catechol oxidase activity, and rate of browning of slices.

Good correlation between browning and O-diphenyl content was found and some correlation between browning and enzyme activity at pH 7.3. Purification of enzymes present in apples, peaches, and apricots is in progress. Several of the enzymes isolated from peaches were studied for their activity, their inhibition by various compounds, and their pH optima.

2. Cell-Wall Structure and Texture. A research grant to Harvard University on cell wall structures has been concluded. An organization of cell cytoplasm that appears to be associated with active cell-wall production has been demonstrated with the electron microscope by applying special fixing techniques developed at the Harvard laboratory. The grantees were able to show that microtubules are always found directly below zones of heaviest cell-wall deposition and that they govern the orientation of cellulosic microfibrils along the wall. This concept of the mechanism of cell-wall formation is entirely new. Cell walls vary widely in different fruits and different parts of fruit, and play an important role in the texture of fruit, drying rates, and stability in freezing and thawing.

Investigations on the formation of hemicellulose as plant cell-wall constituents are being conducted at the National Taiwan University in Taipei, Taiwan, China, supported by P.L. 480 funds. Texture of processed fruits and vegetables is largely determined by the chemical and physical properties of polysaccharides associated with cell walls. Bamboo shoot has the remarkable property of withstanding severe cooking conditions without losing crispness. Because of this characteristic the hemicellulose fraction of bamboo shoots is under study. Starch and pectic substances have not been found in the bamboo shoot. Sucrose serves as the reserve carbohydrate in place of starches, and hemicelluloses appear to constitute the bulk of the cell wall polysaccharides. The hemicellulose fraction consists of two polysaccharides, a galactan and a xylan. The xylan is more closely associated with cellulose than is the galactan, and no proteinaceous material appears to contribute to textural quality in the cell wall fraction. The absence of water-soluble polysaccharide in bamboo shoots appears to have a great bearing on the textural quality. However, the absence of intercellular cementing polysaccharides, such as pectin, raises the question of what material or force keeps the integrity of texture even under severe heating conditions. The chemical and physical properties of hemicelluloses, the biochemical mechanisms by which they are synthesized, and the nature of their association with cellulose are being studied to learn the reason for the textural quality of this product.

3. Pioneering Research. Within the Western Utilization Research and Development Division, a Pioneering Research Laboratory conducts basic research to discover, identify, and characterize the enzyme-substrate systems responsible for formation and disappearance of plant constituents and structures.



Ethylene Metabolism in Plants. Last year this Laboratory reported that the metabolism of either labeled ethylene or labeled acetylene results in the formation of radioactive benzene and toluene. It became apparent that benzene and toluene occur, as such, in mature but unripe avocados. Now fruit has been exposed to small amounts of  $C^{14}$ -labeled benzene and toluene. Both of these hydrocarbons were rapidly metabolized. A small portion of both were metabolized all the way to  $CO_2$ . Volatile and nonvolatile metabolites were formed from the benzene and toluene along chemical routes similar to those observed with ethylene and acetylene. When fruit was exposed to unlabeled benzene and toluene, large concentrations in the surrounding atmosphere and long exposure times were required to produce any visual effect on the fruit. Again, rapid metabolism of these hydrocarbons in avocado was indicated. Work will continue on the separation and identification of products formed in fruit exposed to benzene and toluene as well as to ethylene, so as to elucidate the mechanism by which ethylene exerts its effect in the acceleration of ripening.

Enzymatic Mechanism for Replication of DNA. Information for the biosynthesis of enzymes resides in informational molecules, which are nucleic acids, particularly deoxyribonucleic acid (DNA). This compound is present in the nucleus of all living cells. Since all enzymes for which DNA is coded are not made all at the same time, a mechanism must exist by which only those enzymes needed at a particular point in the life cycle of a cell are made at the right time, and the synthesis of those enzymes not needed is prevented. Good candidates for the agents that suppress transcription of information from DNA are the histones, basic proteins associated with DNA as complexes called "nucleohistones." Not all of the DNA is so complexed. From our current work it appears that the transcription of information for making enzymes can be accomplished only by that part of the total DNA which is not complexed as nucleohistone.

In addition to transcribing information for making enzymes, DNA must also transmit this information to succeeding generations. It does so by replicating itself. Last year we reported that nucleohistone as well as free DNA could serve as a template for the replication of DNA by an enzyme system of bacterial origin. Now for the first time DNA polymerase has been detected in higher plants, in the sprout (seedling) of the mung bean (used commercially in Chinese foods). The enzyme appears to be concentrated in the roots. Its activity on a fresh weight basis is about one-tenth that of bacteria. Thus it appears, as predicted, that the enzyme is made in response to the DNA requirement of the cell.

Enzyme Localization in Plant Cells. Characteristics such as flavor and texture are built into fruits and vegetables by a series of organized enzyme

reactions. In order to control desirable characteristics as well as to eliminate undesirable ones, basic information on the cell mechanisms involved in the induction and repression of enzyme formation is needed. To approach this problem, work has begun on the localization and changes in enzymes which occur in plant tissue cultures (from tobacco plants) where it is possible to work at the cellular level. Cells are propagated by transferring aliquots of an old culture to fresh medium where they continue to grow and divide.

Certain morphological changes occur during growth of a culture, most notably a rapid initial proliferation of new small cells which eventually enlarge as the culture ages. In addition, we find changes in the localization and distribution of enzymatic activity in the cells which are independent of morphological changes at the light microscope level. Thus, for example, certain oxidases (cytochrome oxidase and peroxidase) and dehydrogenases (succinate, malate, glutamate, glucose-6-phosphate, ethanol) are characterized by a shift in localization pattern during the period between inoculation and senescence of the culture (about 2 weeks). Specific enzymatic activity has been shown to rise and fall in cyclic patterns during the growth period.

Patterns of activity are temperature-dependent. Thus, for example, growth cell morphology, enzymatic activity, starch storage capacity, and mitochondrial size and shape are altered by changing the growing temperature from 25° to 35° C. Electrophoresis studies of peroxidase activity from cells grown at 25° and 35° suggest the possibility of an altered enzyme or two different peroxidase enzymes, one operating at 25° and one at 35°.

These studies have demonstrated the unique and special sites in plant cells where enzyme activity occurs and some of the factors that influence the level of activity. From this kind of information it may one day be possible to optimize the kind of enzymatic activity desired and, moreover, to predict activity based on appropriate selection of conditions. Accordingly, this work is being continued.

Biochemistry of Plant Steroids and Related Polyisoprenoids. In cooperation with the Division of Biology of the California Institute of Technology, the enzymatic synthesis and interconversion of plant steroids and related compounds, as well as the chemical reactions of these substances, are being studied with special attention to the manner in which these compounds may function as plant hormones.

Steviol, a diterpenoic acid, occurs in the form of its glucoside, stevioside, in a South American shrub. Stevioside is the sweetest natural compound known. In the past steviol has been tested for biological activity in plants. In one plant, a mutant, it exhibited gibberellin-like activity, thus suggesting that this mutant might possess the ability to convert steviol to a gibberellin. In the present investigation, this possibility was shown to be true.

To pursue this research it was first necessary to have radioactive steviol. The tracer steviol was obtained by feeding Stevia plants radioactive acetate. Strangely enough, feeding labeled mevalonic acid failed to produce labeled steviol, which is counter to the known biosynthesis of other terpenes, and suggests the existence of another enzymatic system for terpene synthesis.

The tracer steviol was converted to a gibberellin by incubating it with a Fusarium mold. The resulting compound was effective on many plants. Isolation of the new gibberellin and characterization thereof showed it to be different from nine known gibberellins. Its precise chemical nature has not yet been established; research is continuing.

Conflicting reports have appeared concerning the occurrence of the female sex hormone, estrone, in palm seeds. In the present research, estrone has now been isolated and identified, not only in seeds and pollen of the date palm but also in pomegranate seeds.

In addition, cholesterol has been isolated in crystalline form from the date pollen. This is the second time this research group has isolated cholesterol from a plant source. Previously, it was thought to occur only in animal tissues.

We have also shown that cholesterol is a precursor of many plant steroids. It is converted in certain plants to the sapogenin, diosgenin, as well as to the latter's analogue, kryptogenin. It also functions as a precursor of plant steroidal alkaloids like tomatidine and the Holarrhena alkaloids, hola-phylline and holaphyllamine, as well as the female hormone, pregnenolone.

Like cholesterol,  $\beta$ -sitosterol, the most widely distributed sterol of higher plants, is formed biosynthetically from the triterpene squalene.

The relationship between gibberellins and anti-gibberellins (plant-growth retardants) has received some attention. The latter have been presumed to function via interference with the synthesis of the former. This mechanism has been confirmed by use of the fungus Fusarium. Cultures of this fungus convert mevalonic acid (a biosynthetic precursor of many isoprenoids) to gibberellins in the absence but not in the presence of the anti-gibberellins.

The study of the enzymatic synthesis and interconversion of plant steroids and related compounds will be continued with special attention to their possible role as plant hormones.

Chemical Alteration of Enzymes. Chemical modification of pure crystalline enzymes in order to locate active regions in protein molecules and to determine relationship of molecular structure to enzymic activity has been investigated by a collaborator with the Pioneering Research Laboratory whose research was supported in part by a grant from the National Institutes of Health.



As previously noted, the acetylated chymotrypsin obtained by directly acylating the enzyme differs markedly from that resulting from activation of already acetylated chymotrypsinogen. In the latter case, the esterolytic activity is greatly enhanced over that of the directly acetylated protein and is usually much greater than the activity of the original (unacetylated) enzyme; the proteolytic activity is not correspondingly increased. Recently, another difference has been observed. The pH optimum of the directly acetylated enzyme is markedly higher than that of the original or the indirectly acetylated protein.

In view of the importance of the subject, considerable attention has been given to the degree of acetylation obtained with chymotrypsinogen. The degree of reaction was determined through the use of radioactive ( $C^{14}$ ) acetyl. As might be expected, the quantity of acetyl introduced can vary widely, with corresponding variation in the properties of the acetylated product.

All this bears directly on the important question of whether the increased stability at high pH and the greater esterolytic activity of the indirectly acetylated protein is attributable to the acylation of some particular group or center, or simply to steric hindrance, which in this case would prevent the protein from rapidly changing its shape. The latter seems more probable, but the question is not settled, albeit very important to anyone hoping to find ways to "modify" an enzyme action.

This work is being discontinued because of the death of the collaborator.

#### D. Microbiology and Toxicology

1. Botrytis Bonification of Wine. Desirable flavors were developed in Thompson seedless grape juice and wine by submerged culture growth of Botrytis cinerea and by addition of Botrytis extract. Favorable comments were received when the Northern California Section of the Institute of Food Technologists was served a very inexpensive white wine that had been improved by addition of Botrytis extract. Bonification of Thompson seedless grape juice concentrate could open a considerable market for this commodity in Europe, which is presently short of inexpensive good-quality grapes. Thompson grape concentrate is used extensively in wine manufacture in areas outside of California where local grape production cannot meet the needs of the industry. Our results indicate the distinct possibility of Botrytis enzyme mixture being used to upgrade quality of white American wines. Cooperative work is being done with the wine industry to demonstrate the effects of Botrytis on larger fermentations than can be conveniently processed in our laboratory.

2. Mold Contamination of Fruit Products. The fungus Byssoschlamys fulva resists heat treatments normally used in preservation of some fruit products, and spoilage outbreaks of this mold have been reported from Western Europe. Heat-resistant fungi, some tentatively identified as Byssoschlamys,

have been found on grapes as they are brought from the vineyard to the crusher, but only at a very low level of contamination. Most appear to be removed with the pomace, since they are not commonly found in the juice. In thermal-death-time cans inoculated with large quantities of Byssochlamys, survival time exceeded 62, 25, and 10 minutes at temperatures of 190°, 194°, and 198° F., respectively. These studies were conducted in collaboration with the National Canners Association's Western Laboratory in Berkeley, California.

#### E. Technology--Process and Product Development

1. New Processes and Products. "Osmovac" dehydration of fruit is terminology we apply to a new process in which about 70% of the moisture in fresh fruit is removed by osmosis, and the remainder (down to less than 5%) is removed by vacuum dehydration. Small whole fruit or cut pieces of fruit are covered with dry sugar or a concentrated syrup. Water and other small molecules are drawn out of the fruit into the sugar or concentrated sugar solution. As dehydration proceeds, the sugar solution becomes more dilute and the solids in the fruit become more concentrated. Eventually the rate of moisture transfer by osmosis decreases, and the fruit is then finish-dried by other means. The second stage may also be carried out by conventional air drying. With vacuum dehydration, a crisp porous texture is retained making the product quite suitable for use as a confection or for incorporation with dry breakfast cereals. Products finish-dried at atmospheric pressures could be useful as ingredients for a large number of formulated products. Because high temperatures are not involved in this dehydration method, heat damage and loss of volatile flavors do not occur. Small amounts of the flavor components are lost into the syrup in the osmosis step. This byproduct syrup has a very dilute fruit flavor. Its use in canning fruits and in ice cream or frozen dessert formulas is feasible. A wide variety of fruits has been prepared in the laboratory by the Osmovac process. Color and flavor retention are exceptionally good. The sugar protects the color of cut fruits so that sulfur dioxide need not be added during dehydration; Osmovac dried fruits maintain their color in storage without aid of sulfur dioxide or other antioxidants. The Osmovac process has been applied with success to whole and sliced strawberries; pitted sour cherries; sliced bananas, apples, peaches, pears, pineapples, nectarines, plums, and papayas; and halved or sliced apricots. Enthusiastic response has been obtained for the quality of these products. Flavor stability during storage, engineering and economic considerations, and applications to other fruits are remaining tasks.

Aromagram flavor analysis to compare commercially freeze-dried strawberries with Osmovac strawberries showed a better retention of volatile components in the Osmovac product, confirming sensory evaluations comparing the products.

High-quality instant applesauce flakes which reconstitute in cold water were made from six varieties of apples with a modified atmospheric double-drum dryer. Vapors evolved during the drying were rapidly removed both from above and below the drums, and a jet of chilled air was directed at the product

film on the drums just before the films reached the doctor blades. High-sugar products are sticky at high temperature, thus the chilling was necessary so the film could be drawn from the drums by a variable-speed reel which metered the dry product from the drums and permitted control of final flake thickness. The product, being hygroscopic, was collected in a low-humidity chamber. Potential applications of this process, which can be used for other fruit purees as well, include production of instant fruit sauces, preserves, and apple butter, use in bakery mixes and confections, and compression of the dried products into flakes or disks suitable for packaging with dry breakfast cereals.

We are applying microwave heating in developing new processes and products. Energy transfer via microwaves provides rapid and uniform heating throughout the exposed material. Because the equipment is relatively expensive, we are combining microwave effects with conventional heat for blanching, drying, cooking, and warming. Large continuous microwave-generation equipment is now available commercially, and a new technique, microwave puffing, is available to produce quick-cooking products. Fruit and vegetable products are prepared by conventional dehydration techniques until the moisture content is reduced to 50% or lower. The products are then placed in a microwave processing chamber where a portion of the residual moisture is rapidly vaporized, which puffs the product. The product may then be dried to the desirable final moisture content by microwave drying or by other processes. Examples we have worked with are apple segments, potato slices, precooked beans, and precooked rice. In addition, we have used microwave heating to blanch large vegetables or large pieces of vegetables that are difficult to blanch by hot water or steam. Corn on the cob and Brussels sprouts have been blanched adequately with less total exposure to heat, and superior frozen products have been produced.

"Wurvac" is a term we have applied to a new process for stripping aromas from fruit and recovering them for reincorporation into concentrates. This method was found to be most suitable for obtaining heat-sensitive aromas, such as those from citrus and peaches, because it enables their recovery at low temperatures under vacuum. Loss of aroma components into the vacuum system, experienced in most existing methods, is prevented by absorbing the aroma in the sealant of a liquid-seal vacuum pump. Orange juice aroma solution contained aroma-contributing compounds not found in peel oil. Gas liquid chromatography was used to check the efficiency of the process and the quality of final products. Aroma solutions were evaluated by using them instead of fresh juice cut-back in citrus concentrates and for flavor potentiation of fruit drinks. Evaluations using both sensory tests and gas chromatography showed the aroma solutions to be of exceptionally high quality. Storage stability of aroma solutions obtained by Wurvac was found to be good even in cold storage above freezing. Much higher concentrations of fruit aroma can be achieved by Wurvac than by previous essence recovery systems.

Liquid carbon dioxide under pressure at room temperature has been found to be a selective solvent for fruit aromas. Preliminary experimentation was



conducted on recovery of apple aromas. Fruit constituents such as sugars, acids, inorganic salts, etc., are insoluble in liquid carbon dioxide and so are not removed. The selectivity of the solubility permits the extraction of aromatic materials in essentially pure form. Apples, pears, and other fruits have been processed in a continuous system at 25° C. and 65 atmospheres of pressure. The extracts obtained are comparable in flavor to the original, as evaluated by both sensory and gas chromatographic techniques.

Preliminary investigations on use of osmosis for concentrating liquid food products have been promising. Concentration at atmospheric pressure can be accomplished by the osmotic pressure differences between the low concentration fruit juice on one side of a dialysis membrane into a suitable concentrated solution on the other side. Water and very little else moves from the fruit juice into, typically, a concentrated solution of inorganic salt of some type.

Reverse osmosis is a process in which pressure considerably in excess of osmotic pressure is applied to the high-concentration solution so that the water moves through the dialysis membrane in the direction of the low-concentration solution. This pressure-induced reverse flow has been neglected in the past because very strong semi-permeable membranes were not available. Pressures up to 2500 lbs. per sq. in. are required for some products. We have prepared porous tubular membrane supports, following general methods developed for desalinization, and developed equipment for reverse osmosis concentration. Apple and orange juices have been concentrated to about 40% solids by pressures up to 2500 lbs. per sq. in. The rate of water removal decreases with increased concentration of soluble components. Some potential uses for reverse osmosis in agriculture are the concentration, with minimum flavor loss, of fruit juices and beverages, economic water recovery from brackish irrigation run-offs and processors' effluents, low-temperature concentration of liquid foods, concentration and salt removal from molasses and whey, concentration and acid removal from citrus juices and syrups, concentration of sugar beet thin juice, etc. Studies of these new processes will continue.

2. New Dried Fruit Products. Prunes and other dried fruits can be made more succulent and attractive by hydrating to very high moisture levels. A treatment with 10% sorbate solution preserved prunes at 55% moisture content. Diethyl pyrocarbonate at 0.05% concentration inhibited growth of molds and yeasts on prunes at 35% moisture. Combinations of reagents may be more effective than high concentrations of single reagents. Continued studies on mold-controlling agents for high-moisture dried fruit is of great interest to the industry.

Commercial interest has been developing in the non-setting raisin paste made by our heating process. Untreated ground raisins set to an intractable hard mass within a day or two after grinding, but a simple heat treatment after the raisins are ground imparts a soft pliable texture to the raisin paste, and the paste remains soft after 30 days' storage. In this form it is useful to the bakery trade in making cookies and pastries. Without the treatment, raisin paste could not be used as an ingredient for such products.

3. Grape Products. Low-alcohol and high-alcohol fermentations are being investigated to broaden wine product lines as a basis for increasing markets for grapes. Low-alcohol wines were prepared and treated to provide a beverage intermediate between grape juice and the usual table wines. High-alcohol fermentations, without sulfur dioxide, carried out on Thompson seedless, Sauvignon Blanc, and Semillon musts and concentrates yielded high-quality wines. Samples of these wines were presented to the Wine Institute Technical Advisory Committee for evaluation at a recent meeting. The high-alcohol fermentation produces wines that are easier to preserve and provides a dessert wine free from the heat-induced furfurals that arise from adjusting alcohol content upward with grape brandies. This non-fortified dessert wine is a completely new class of wine, but it does meet the standards of identity for the usual dessert wines.

Investigations are being conducted under contract at the University of California at Davis on new and improved methods for separating juice from grape pulp and seeds. A number of grape varieties were processed to show the effects on the skins of various heating times with fixed fermentation time. Heat was applied rapidly for 5, 10, 15, or 20 seconds. Taste scores on the different wines after 5 months of aging showed that heat-treated wines were superior or equal to unheated controls. For each variety, there was an optimum heat treatment. The amount of color extracted originally and retained during 3 months' aging was higher in samples that had been heated longer. Extracting color from skins of red grapes during fermentation is difficult to achieve without adversely affecting the flavor and red pigment stability. Very rapid heating for short periods appears to provide improved extraction and color stability compared with methods previously used.

A three-stage counter-current system was developed at Davis to recover sugar for fermentation from grape pomace. Earlier methods of washing the sugar from the pomace with water provided solutions that were extremely dilute and inefficient in later fermentation and recovery of alcohol. Allowing the fermentation to take place on diluted pomace led to difficult disposal treatments of the resultant still slops when the alcohol was recovered by fermentation and distillation. The new counter-current extraction method is more satisfactory in that adequate recovery of sugar from the pomace can be obtained in suitably concentrated solutions which can then be fermented to recover alcohol.

Investigations are conducted under contract at the New York Agricultural Experiment Station in Geneva on the chemistry of undesirable precipitates that form during the production and storage of wines. A thorough review covering the literature from America and Western Europe has been made of the use of protein tannin reactions for fining of fruit juices and wines. Preliminary experiments with model systems using tannin-gelatin and isinglass-tannin are being conducted to obtain data on the removal of tannins. The conditions for development of a tannin-protein haze in the solution is being studied and the research includes removal of haze by filtration as well as by fining.

4. Processing Quality of Northwest Fruits and Berries. Cooperative research with the Washington Agricultural Experiment Station continues. Seven varieties and 64 hybrid selections of strawberries, 7 varieties and 28 selections of raspberries, and 11 varieties of blueberries from the 1965 harvest were processed. Most of the selections were from the third year screening of 4,000 seedlings planted in 1963. Several promising selections from the 1960 plantings were re-examined. Of particular interest to plant breeders are selections that show superior tolerance to winter damage, which has been very harmful to berry growers in Washington and Oregon during the past several years. As a result of our examinations, more than half of the 64 strawberries and 28 raspberry hybrids evaluated have been discarded for lack of desired quality. The Hood variety, which was introduced by Oregon State University and the United States Department of Agriculture in Oregon last year, was found to be outstanding for preserves and freezing. Clone B-22, a virus-free clone of the Northwest strawberry, was judged equal to the current commercial Northwest variety for freezing.

Recipes for pectin-gel candies made with fresh-fruit concentrates have been distributed; they are creating some commercial interest in the Pacific Northwest.

The ranges in composition of Concord grape and grape juices were studied and some of the factors affecting composition determined. Maturity, seasonal weather variations, crop load, and soil fertility are the dominant production variables that affect composition. The composition attained by grapes appears to be more closely related to cumulative solar radiation than to cumulative heat units. Only in seasons of unusually low solar radiation do vines bearing crop loads exceeding 10 tons per acre fail to mature grapes to 16% total solids in central Washington. With similar crop loads, vines on more fertile soil product grapes of lower soluble solids and higher acid content than vines on less fertile soil. Seasons of low solar radiation result in grapes with higher acid content than in seasons of high solar radiation. Acid content more nearly reflects climate during the growing season than does soluble solids content because the acid content does not appear to be affected by crop size. The amounts of color, methyl anthranilate and tannin in grapes varied considerably from season to season, but production variables affecting these constituents were not apparent. Differences in composition of grapes affected flavor of juices mainly in sweetness and tartness. Adjustments of soluble solids and acids comparable to those used in preparing frozen concentrated sweetened grape juice evened out differences in original juice flavor.



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## AREA NO. 8. POTATO UTILIZATION - FOOD

Problem. The potato industry, faced with a continuing decline in the consumption of fresh potatoes, is becoming more and more dependent upon the development of new and improved processed products to maintain markets and to avoid recurring economic disasters. Crop perishability, supply fluctuations, and the inelasticity of demand result in wide swings in price with even slight surpluses. In producing areas having a substantial processing industry, growers can moderate depressive lows by contracting with processors prior to harvest. However, in many important potato growing areas where processing has not yet developed, growers are still vulnerable and the situation is exaggerated by the mounting competition from processed potato and other food products. A continuing improvement in processed potato products is clearly required if processing is to expand fast enough to offset the progressive decline in use of fresh potatoes.

To improve the quality of processed potatoes, ways must be found to eliminate the stale, earthy, rancid, green, and warmed-over flavors that are sometimes encountered in potato products, including dehydrated mashed potatoes, dehydrated diced potatoes, frozen french fries, frozen patties, and potato chips, and to retain the desirable natural flavor of freshly cooked potato. Research methods recently developed offer an opportunity to isolate and identify the constituents responsible for the natural flavors and the off-flavors, to develop rapid and sensitive analytical methods for their measurement, and to determine the raw material factors controlling formation of the various desirable and undesirable constituents in the fresh potato.

Further improvement in the texture of potato products is also needed. Fundamental histological and chemical investigations could reveal the causes of differences in the texture of potatoes, as a basis for developing improved processing methods.

Enzymes play a great part in the entire compositional pattern of the potato, not only the constituents responsible for flavor, off-flavor, color, and texture, but also those responsible for disorders such as black spot. Black spot causes severe losses, both to those who market potatoes fresh and to those who process potatoes, because trimming costs are sharply increased and yields reduced. Increased knowledge of enzymes is needed as a basis for solving the black spot and similar problems, for increasing use of potatoes by reducing costs, and for improving quality of both fresh and processed potatoes.

## USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, basic and applied research on potato products is conducted at the Division headquarters at Albany, California, by a grant at Davis, California, and by



grant funds under P.L. 480 in Sweden. The chemistry of potato flavor and the compounds involved in deterioration of potato products are studied to provide a basis for new and improved potato processes and products. Histochemical studies are conducted to elucidate factors involved in the texture of potato products. Basic investigations on the enzyme systems involved in potato product discoloration and the mechanism of rancidity development are in progress.

The Federal program of research in this area totals 4.9 scientist man-years including the equivalent of 0.7 scientist man-years for a research grant. Of this number, 0.7 are assigned to chemical composition and physical properties; 1.2 to flavor; and 3.0 to technology--process and product development. In addition, the Division sponsors two research grants under P.L. 480.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 15 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition and Physical Properties

1. Potato Components. Research has been initiated under a grant to the University of California at Davis to provide new basic information on lipid synthesis, lipid breakdown, and phospholipid transformation as they relate to rancidity in stored dehydrated vegetables. The lipids of vegetables are subject to oxidative degradation, a serious form of deterioration in potato products. This new project will provide insight into the mechanism of formation of fats in living vegetable tissues and, in the long run, it may provide us with ways to control fat composition in processed foods. Biochemical reactions stimulated when discs of potato tissue are aged in distilled water are under study. Under these conditions, linoleic acid, which is the major fatty acid of potato lipids, is formed from monounsaturated oleic acid.

These studies also utilize vegetables other than potatoes to determine the synthesis of lipid components. Either light or a strongly reducing substance stimulates the incorporation of acetate into the chloroplasts of butter lettuce. The light-induced stimulation incorporates the acetate into nonvolatile long-chain fatty acids, whereas the reducing substance converts the acetate into a lipid-like volatile substance that has not yet been identified. Acetate, which is a precursor in fatty acid biosynthesis, is present in spinach and lettuce chloroplasts. The origin of acetate for this purpose has not been discovered but some of the earlier postulations have been tested and found wanting. A heat-stable protein that is involved in the fatty acid biosynthesis, acyl carrier protein, had previously been isolated from microorganisms. In this work a protein that is different in molecular weight and thiol content has been isolated. It has the same function as the protein isolated from a bacterium.

Two P.L. 480 grants have been made to the Swedish Institute for Food Preservation in Gothenburg. Under one of these the role of metals in enzyme action is being investigated. The purpose is to elucidate the relation between chemical structure and catalytic activity of enzymes. Metal-requiring enzymes have been chosen as particularly suitable objects of investigation because the metal provides a natural label of the portion of the protein directly involved in the reaction. The molecular architecture, the amino acid composition, and the physical properties of five different enzymes are being studied. Laboratory procedures and methods of analysis have been advanced. For the enzyme 3-phosphoglycerate kinase at high concentrations of divalent magnesium ions, four binding sites exist. At low metal ion concentration there are only two. Carbonic anhydrase, alkaline phosphatase, laccase, and tyrosinase are the other enzymes being studied.

With the other grant, the relationship between oxygen pressure and the rate of oxidation of fats found in dehydrated vegetables is being studied by means of model systems. The autoxidation in such a system is related to that found in storage deterioration of dehydrated potatoes. Addition to the model system of certain pro-oxidative metal ions, divalent copper and manganese, reduced the rates at which oxygen was consumed at low oxygen pressure. The effect of certain antioxidants was prolonged if the oxygen pressure was low. The equipment modifications which have been made to improve the study of oxygen utilization at low oxygen pressure are considered a major advance and applicable to a wide field of research.

## B. Flavor

1. Flavor of Potato Components. One of the common defects of dehydrated potatoes, particularly potato flour, granules, and flakes, is a rancid off-odor that results from the oxidation of the small amount of fat that is present. Positive prevention of rancidification has not been possible on a commercial scale through the use of antioxidants or nitrogen packing, although storage stability has been materially enhanced by such devices. The concentration of hexanal in the vapors over hot reconstituted potato granules has provided a good index of the amount of oxidized aroma. Non-enzymatic browning or scorching of dehydrated potatoes can occur rapidly in the final stages of dehydration if temperature is not carefully controlled. Browning occurs, but more slowly, during storage of the dry product. High storage temperature increases the rate of nonenzymatic browning. The concentration of certain branched-chain aldehydes in the vapors over hot reconstituted granules is a measure of the amount of scorching, although it has not been shown that these compounds themselves are responsible for the off-odor. Volatile compounds identified as emanating from both fresh and stale potato chips include acids, saturated and unsaturated aldehydes, ketones, and mercaptans, among others. Preliminary evidence indicates that carbonyls play important roles in the aroma of potato chips.

We have also studied the aroma of boiled potatoes in an attempt to discover something of the chemistry of cooked fresh potatoes. The sulfur-containing

volatiles from cooking potatoes were analyzed by gas chromatographic and chemical techniques. Methyl mercaptan and dimethyl disulfide were the major sulfur-containing compounds detected in the vapor. In smaller amounts were ethyl mercaptan, dimethyl sulfide, methyl ethyl disulfide, and methyl isopropyl disulfide. Several other compounds were detected in trace amounts. Hydrogen sulfide was produced in relatively large amounts and over extended periods during cooking of either fresh or dehydrated potatoes. Because these sulfur-containing compounds have such a low odor threshold, we believe they are important factors in potato flavor.

### C. Color, Texture and Other Quality Factors

1. Color and Texture of Frozen French-Fried Potato Products. Nearly a billion pounds a year of frozen french-fried potatoes are produced. About 80% are used for restaurant and other institutional food services. Nevertheless, french-fried potatoes made from the frozen product do not meet specifications of certain large restaurant operations because color and textural quality is frequently not up to the standard achieved with fresh potatoes. We have developed a method for measuring objectively the color changes of fried potatoes, which will be useful in research aimed at improving the quality of frozen potatoes.

Microscopic studies have revealed that changes in texture occurring as a result of freezing, thawing and refrigerator storage are related to changes in the properties of the gelled starch. Starch retrogradation occurs, and consequently the water-holding capacity of the starch gel is altered. This influences interior mealiness but its relation to the crispness of the outer surface of french-fried potatoes requires further study.

### D. Technology--Process and Product Development

1. Fat Uptake During Frying of Frozen and Thawed French-Fried Potatoes. Managers of many small drive-in restaurants have adopted the practice of storing frozen french-fries in the refrigerator where temperatures range from 35° to 50° F. We have investigated the quality changes that occur during a several-day holding period at 34°, 45°, and 55° F. An important observation was that the amount of fat absorbed by french-fries increases as a consequence of their being thawed and held in storage prior to finish-frying. In one experiment, potatoes that were finish-fried starting with the products frozen had a fat content of 8-9% as prepared for serving, those thawed and held at 35° F. had up to 13% and those that had been stored at 45° and 55° F. contained even more fat.

2. Improving the Stability of Potato Granules. A number of chemical additives are permitted in the manufacture of dehydrated potato granules. Common materials that aid stability are sulfur dioxide, butylated hydroxyanisole, butylated hydroxytoluene, and glycerol monostearate. High moisture levels inhibit oxidation but accelerate browning. Sugar or corn syrup solids added to potato granules of high moisture content protected the



granules to a significant degree against conditions that induce browning. Chelating agents, such as sodium acid pyrophosphate and ethylenediamine tetra-acetic acid, were applied to potato granules prior to dehydration and found to limit the evolution of hexanal from freshly reconstituted granules. The hexanal test is indicative of oxidative rancidity.

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## AREA NO. 9. VEGETABLE UTILIZATION - FOOD

Problem. Vegetable crops, in general, are perishable and seasonal and thus are subject to supply and price fluctuations to the disadvantage of the agricultural economy. In order to expand markets and stabilize prices, new and improved processed products are needed that will be desirable to the domestic and foreign consumer from the standpoint of quality, convenience, stability, nutritive value, safety, and cost. The quality of processed vegetables and the economy of their processing have not improved rapidly enough to increase or even maintain the relative position of vegetables in the American diet, or to increase substantially their contribution to the export trade. The consumption of dry beans and certain other vegetables is limited by the fact that they cause flatulence.

New easy-to-prepare vegetable products are needed, particularly from such commodities as dry beans and peas, which now require hours to prepare. The severe heating required to sterilize low-acid foods, which include most vegetables, seriously impairs the quality of canned products. The stability of all kinds of processed vegetables needs to be improved so that quality and nutritive value will be better preserved during storage and distribution. The safety and effectiveness of new chemical additives, needed to improve the quality and stability of processed vegetables, must be established. Processing operations need to be modified to cope with special problems arising from the trend toward mechanical harvesting of many vegetables. Better methods of removing residues of agricultural chemicals from vegetables for processing are urgently needed, as are procedures for decontaminating vegetables exposed to radioactive fallout. Of vital importance is research to reduce the costs of processing in order that the farmer may receive a larger share of the consumer's dollar.

Applied research on these practical problems must be supported by a strong program of basic research on the chemical constituents of vegetables responsible for flavor, color, and texture; on the reactions these compounds undergo before, during, and after processing; on constituents having biological activity; on the microscopic structure of vegetables and vegetable products; and on the micro-organisms which cause spoilage or loss of quality in these products.

### USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic research on vegetables and the application of science to new and improved products and processes is conducted at the Division headquarters at Albany, California, in field stations at Pasadena, California and Puyallup, Washington; by contracts and grants at Urbana, Illinois, East Lansing, Michigan, and Davis and Berkeley, California; and by grants under P.L. 480 in Finland, India, France, and Sweden. Fundamental studies

are conducted on the chemistry of vegetable flavors and vegetable pigments, the mechanism of heat resistance in bacterial spores, the composition of dry beans as related to cooking quality and flatulence-producing characteristics, the factors affecting deterioration of dehydrated vegetables, and the microbiology of raw vegetables for processing. Applied research is conducted to develop new and improved products including high-quality concentrated and dehydrated products and products of improved convenience; to improve processing methods, including freezing; to evaluate new processing varieties; and to develop methods for removing radioactive fallout.

The Federal program of research in this area totals 30.9 scientist man-years, including four scientists whose salaries are provided (two each) by the California Lima Bean Advisory Board operating under a State Marketing Order and the United States Brewers Association, and two summer student employees whose salaries are supplied by the National Frozen Food Packers Association; and four contracts and grants equivalent to approximately 2.3 scientist man-years per year. Of the total, 2.4 are assigned to investigations on chemical composition and physical properties; 8.0 on flavor; 4.9 on color, texture and other quality characteristics, and 9.2 on technology--process and product development. In addition, the Division sponsors three grants under Public Law 480 on basic research.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 48 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition and Physical Properties

1. Composition of Dry Beans. The search for the fraction of dry beans that is responsible for flatulence was advanced by improvements in methods of gas collection and analysis. Breath analysis has been correlated with analysis of flatus, a correlation useful in experimental work. Breath hydrogen increases and decreases in a pattern identical with the fluctuations of flatus volume for 12 hours after a subject has eaten. In a cooperative study with the Department of Nutritional Sciences at the University of California at Berkeley, flatus and breath gas analyses were made on 12 human subjects to evaluate foods developed for the Gemini space flight program.

The new white Ventura variety of Lima bean was analyzed as to variation in its flatus-producing character during the growth and maturation of the seeds. The flatulence factor was present as much as three weeks before the beans reached a green-mature stage. Sprouted California small white beans produced the same degree of flatulence in human subjects as ungerminated beans. Lima beans of the Fordhook variety eaten in either the succulent green stage or the dry mature stage were relatively non flatulent.

The sugars, stachyose and raffinose, which were found in the flatulence-producing chemical fraction of dry beans, were fed in synthetic meals to six human subjects in cooperative research at the University of California. All six subjects showed a rise in breath hydrogen but no significant



increase in flatulence. These complex sugars are not readily metabolized by man, but they may serve as a substrate for intestinal microorganisms which produce one of the major flatus components, hydrogen.

Contract work at the University of Illinois on flatulence-producing properties of dry beans shows that anaerobic bacterial fermentation of some component(s) of beans is in part responsible for gas formation. Bean homogenates also have an inhibitory effect on intestinal carbonic anhydrase (an enzyme that facilitates transfer of carbon dioxide into the bloodstream), and thus they contribute to the accumulation of carbon dioxide in the intestine. Results of this research have not supported the idea that intestinal gas is formed by acidification of pancreatic bicarbonates. The work has further confirmed that stimulation of mechanical activity of the intestines is not a cause of flatulence.

An investigation of the proteins, amino acids, and biologically active components of dry beans is being conducted with P.L. 480 funds at Allahabad University in India. Nitrogenous constituents of defatted bean meal at various pH levels are being extracted with 8 different solvents at 3 different concentrations. The amounts and the nature of nitrogenous constituents extracted by different methods are under investigation. Protein fractions from 2 varieties of common beans were separated and purified and are being characterized.

## B. Flavor

1. Vegetable Flavor Components. Aroma is an important attribute of most foods, as are taste, color, and texture. It has long been possible to measure chemically such taste-contributing constituents as sugars, acids, salts, and some bitter substances. It is also possible to measure color and texture fairly well by objective methods. However, until recent years little progress has been made in measuring the aroma-bearing constituents. Research that we have conducted has advanced the analytical techniques and is producing new knowledge which will be of value in the control of aroma of vegetable products and the development of new products of improved aroma.

Up to 150 parts per billion of hydrogen sulfide was found in the volatiles stripped in an hour from tomato products at 100° C. This is roughly 15 times the odor threshold of hydrogen sulfide in water. However, H<sub>2</sub>S disappears in canned products by reaction with metals, and its overall importance to tomato products is probably much less than that of dimethyl sulfide, which does not disappear in the canned product.

Our research has revealed a substance not previously described in the literature but present in fresh tomatoes and also in considerably larger amounts in asparagus and cabbage. Preliminary studies suggest that the substance may be a short-chain molecule made up of amino acids and containing two or more glutamic acid building blocks.

Tomato powder dried by the foam-mat process is stable in nitrogen at room temperature for a year, but storage life is reduced at higher temperatures. Amino acids were determined and found to react during the storage of dried tomato powder. Such changes in amino acids may be connected with nonenzymatic browning and a consequent loss of flavor quality in tomato powder.

Investigations on organic components in vegetables and fodder plants is supported by a P.L. 480 grant to Nobel laureate A.I. Virtanen at the Biochemical Institute in Helsinki, Finland. Professor Virtanen has been studying the aliphatic sulfenic acids to which the lachrymatory substance of onion, propenyl sulfenic acid, belongs. His group has synthesized not only the propenyl sulfonic acid precursor, propenyl cysteine sulfoxide, but the corresponding lower homolog, S-vinyl cysteine sulfoxide, and the higher homolog, S-butenyl cysteine sulfoxide. Acids from these three compounds are formed from the sulfoxide under the influence of an enzyme preparation made from onions; they have similar lachrymatory effects. Newly discovered sulfur compounds have been isolated from onions and from the seeds of chives. A tripeptide consisting of glutamic acid plus 2 molecules of S-propenyl cysteine was identified. A study on decomposition or elimination of organic disulfide is in progress. The flavor substances of onion which cause off-flavor in milk when onions are fed to cows are disulfides.

The amino acid discovered by Dr. Virtanen to be a precursor of the lachrymatory principle in onions (propenyl cysteine sulfoxide) yielded a good clear magnetic resonance spectrum that established the molecular conformation indicating the trans nature of the double bond. This amino acid has been studied in cooperation with Mr. T. M. Lukes of California State Polytechnic College.

In studying these flavor compounds of onion and related compounds in other vegetables, mass spectrometry is providing a powerful tool for identification of individual components. However, in interpreting mass spectra, we are handicapped by a lack of knowledge of the ways in which molecules are fragmented in the mass spectrometer. To aid in this problem known compounds are synthesized so that their fragmentation patterns can provide insight and comparison with the patterns of unknown substances. When heavy hydrogen is incorporated in specific locations in synthetic compounds, the mass spectra produced yield information that is especially valuable in understanding the patterns of fragmentation.

New studies were initiated to develop an improved method for rapid evaluation of flavor strength of onions and onion products. A test more sensitive than the pyruvate method we developed earlier is desired by processors. Gas chromatographic analysis of onion vapors appears to be potentially useful for the purpose. Onion samples are crushed and the vapor above the sample injected into a capillary column gas chromatograph. Only a low concentration of volatiles appears immediately after crushing but in about 5 minutes peaks begin to appear in the gas chromatogram. The total area of all the peaks in the aromagram then increases markedly until an apparent

maximum is reached after 7 to 8 hours. From then on the peak area of the chromatogram decreases; this indicates a tailing off of aroma strength.

2. Flavor-Bearing Components of Hop Oil. In work supported financially by the U.S. Brewers Association, the volatile constituents of hop oil have been extensively studied using gas chromatographic separations. The compounds are identified by chemical and physical measurements of the isolated fractions. Well over 100 volatile components of hop oil have been isolated and identified, and we are now determining which of these are extracted from the hops and appear in hop beverages in their original form and which are extracted but altered in the brewing process. Hydrocarbons, which make up the major part of hop oil, are not extracted into the water phase and, therefore, probably contribute little if anything to the beverage aroma. The main volatile constituents extracted when hops are boiled in water are free organic acids. The main non-acid constituent appears to be humulene epoxide. Another important non-acid constituent identified by mass and infrared spectral evidence is humulenol, a rearranged product of humulene epoxide.

Determination of the odor threshold of hop constituents indicates that a large number of moderately odorous constituents contribute to the total hop aroma. In the making of beer when hops are boiled with the wort, the higher-boiling oxygenated constituents seem to transfer into the wort most efficiently, whereas the terpenoid hydrocarbons seem to be largely lost. Fermentation appears to change the chemical nature of many of the hop oil constituents that get into the wort, the principal change being the conversion of methyl esters to ethyl esters.

3. Sensory Analysis of Odor Qualities. The scientific study of olfaction has been hampered by much uncertainty about the specific property of a chemical compound that stimulates the sense of smell. The stereochemical theory of odor originally postulated seven odor standards based on descriptions most frequently found in chemical literature and the distinctive common molecular dimensions or reactivities consistent within each standard. Organoleptic judgments have been used to describe odors of chemicals quantitatively in terms of similarity to one of the the seven odor standards. Correlations have been drawn between odor similarity scores obtained by the panel of judges and the dimensional similarities exhibited by silhouette patterns of molecular models of the specific compounds. For each of the following odor classes--ethereal, camphoraceous, musky, floral, and minty--a highly significant linear correlation was observed. This provides strong experimental evidence that the psychophysical basis of odor is primarily molecular size and shape.

For further verification and more precise selection of the odor standards, an experimental procedure was developed using subjects with specific anosmia. People exhibiting specific anosmia cannot detect certain odors or cannot detect them at concentrations readily apparent to most people. It is postulated that a person exhibiting a specific anosmia would be lacking in



olfaction receptor sites having the capability of detecting molecules of a specific size and shape. Theoretically, the particular size and shape that he cannot detect would be considered a primary odor, approached by other compounds that are approximately the same size and shape. About 2% of the population have a specific anosmia for isobutyric acid (rancid, sweaty odor). These anosmic persons can detect isobutyric acid only in concentrations about 50 times higher than the threshold for normal observers. In comparisons using straight-chain acids with 1 to 10 carbon atoms in the molecule isobutyric-acid anosmics exhibit roughly the same degree of anosmia for the acids with 4 to 7 carbon atoms. About 30-fold concentration above normal is required for detection of these straight-chain acids. The ester, isobutyl isobutyrate, appears to be virtually unrelated because the threshold ratio for anosmics is only 2-fold. The highest threshold ratio so far observed was 95-fold for isovaleric acid. This suggests that isovaleric acid must closely approach the ideal molecular configuration for eliciting the "sweaty" primary odor. This basic research is providing a deeper understanding of the relationship of chemical compounds to specific odors and should be especially helpful in our research to produce products of improved flavor.

### C. Color, Texture, and Other Quality Factors

1. Effects of Freezing Rate on Frozen Vegetables. If vegetables are frozen rapidly enough, for example with liquid nitrogen, solid carbon dioxide, or by other means, they can be thawed with very little, if any, cell wall breakage visible microscopically, and they have a texture more nearly resembling that of the fresh vegetables. Test panels were used to seek a correlation between human judgment of green bean texture and tissue damage that could be observed with a microscope. In order to study these factors in greater depth, new equipment for microscopic observation and for freezing under controlled conditions is being prepared. The National Association of Frozen Food Packers is providing salaries for two student subprofessional assistants to work during the summer season on this project.

Five varieties of green snap beans, including Blue Lake and four bush bean varieties, were used to study the effects of maturity and freezing rate on product quality. Three harvest dates and three freezing methods were used (frozen in the package, frozen loose on trays in an air blast, and frozen by immersion in liquid nitrogen). The greatest difference in quality of product was caused by the difference in freezing rate. The liquid nitrogen freezing gave the highest shear press values for all sieve sizes and for all varieties. Changes due to sieve size were not large when compared with changes due to freezing treatment. After the beans were cooked, those that had been frozen in liquid nitrogen were the firmest. Liquid nitrogen freezing tests with other vegetables and fruits showed that texture improvement was possible with asparagus and corn on the cob and that berries frozen in liquid nitrogen did not break down and lose their juice as rapidly after thawing as did berries frozen more slowly.

2. Histology of Dehydrated Vegetables. A common defect in dehydrated vegetables is their inability to rehydrate to their original moisture content, a defect that is particularly serious with onions, which are not blanched before dehydration and which may be merely rehydrated in cold water and eaten without cooking. Contract research on the effects of dehydration on reconstitution characteristics of vegetables is supported at the University of California at Davis. Freeze-dried onions reabsorb more water on reconstitution than do air-dried onions. In the case of freeze-drying, cellulosic crystallinity increased as drying progressed, but volume and texture changes were nil. In onions dried by other processes, not only did cellulose crystallinity increase, but the volume that could be regained on rehydration decreased and the toughness of rehydrated tissue increased.

3. Vegetable Pigments. The effect of dehydration and storage of dehydrated products on yellow pigments was investigated. Two varieties of parsley were selected to serve as models for pigment deterioration. Total beta-carotene content of the dried product that had not been blanched was quite stable; values were slightly higher than for blanched and dried material. Xanthophylls were similar. All-trans beta-carotene decreased after drying. Dried samples were stored for three months under nitrogen and under air at 22° and -18°C. A 10% to 15% loss of total carotene occurred in the unblanched sample during storage in air at 22° C.

#### D. Microbiology and Toxicology

1. Heat Resistance of Spores. The heat required to destroy bacterial spores and sterilize low-acid canned vegetables and other foods seriously degrades color, flavor, texture, and nutritional quality. In order to develop less-damaging processes, we are conducting a long-range basic study on the nature of heat resistance in bacterial spores. We have found that a previously unrecognized ion-exchange property of spores determines their heat resistance. The spores can be sensitized to heat by an acid treatment and this sensitivity persists temporarily when the product is returned to its original non-acid state. Consequently less heat is required to sterilize spores treated in this way. Spores of B. subtilis are resistant to heat when the ion exchange mechanism is loaded with calcium ions. Under acid conditions, hydrogen ions replace the calcium and the spores lose their resistance to heat. A reversible activation of spores of B. stearothermophilus was obtained by exposing the spores to a medium at pH 1.5, and then reversing the spores to dormancy by exposing them to an alkaline solution of calcium ions. A public-service patent on the method has been applied for.

Laboratory trials of reducing heat-processing severity have been made on a variety of commercial low-acid strained foods inoculated ( $10^8$  spores per gram) with the flat sour organism, B. stearothermophilus. After addition to the food product the spores were sensitized to heat by acid treatment, then restored to their normal pH and heated to 240° F. Ratios of heating time required for a million-fold reduction of viable spores in the control-inoculated product to that in the acid-treated sensitized samples were

favorable. For strained carrots, a million-fold reduction of viable spores was achieved in one-eighth of the time required without the new treatment; in sweet potatoes, one-ninth; green beans, one-fourth; beef, one-fourth; beets, one-ninth; chicken, one-fifth; creamed corn, one-half to one-third; squash, one-eleventh; and creamed spinach, one-fourth. The acidification followed by neutralization provides a net addition of 0.2 to 1% sodium chloride to the product, depending upon which product is treated.

It is not contemplated or recommended that any reduction in severity of heating possible by this approach be extended beyond the usual process to prevent survival of the dangerous Clostridium botulinum. However, in commercial practice, heat severities of 2 to 10 times that required to control botulinum are used. Presumably several-fold reductions in heat severity for some products would be possible without subjecting products to danger of botulism.

Ultraviolet absorption spectra have been obtained on dry spores embedded in potassium bromide. These spectra, which are characteristic of calcium dipicolinate, provide evidence that at least part of the calcium and dipicolinic acid in spores are bound together in the form of a chelate. An improved spectrophotometric assay for dipicolinic acid has been developed. It represents a considerable gain in sensitivity and specificity that should be useful in many phases of research on heat-resistance of spores. Separation of spore populations in an aqueous density-gradient preparation has been made, and the fractions will now be studied for their dipicolinic acid contents and differences in heat-resistance.

Microincineration and electron microscopy applied to B. megaterium spores have revealed an organized mineral structure in the spore coat and have demonstrated the gross distribution of minerals throughout the spore. Electron microscopy also revealed the existence of a very thin membranous sac which apparently was released from B. macerans spores that had been dry ruptured. The sacs were relatively scarce in the preparations, and were difficult to detect because of their very low contrast. Nevertheless, they could be recognized with certainty by their distinct and uniform appearance. The membrane appears to be quite smooth without substructure at the highest magnification used and, in some instances, the membranous structure was seen protruding from the partially broken spore coat. The position and size of the structure suggest that it is either an inner layer of the coat or perhaps a separate membrane surrounding the spore cortex.

Basic research on factors that lead to bacterial sporulation are being investigated under a grant made to the University of Illinois at Urbana. Knowledge is being obtained on the biochemistry and physiology of Clostridium thermosaccharolyticum, an organism in which it is hard to induce sporulation. A procedure was developed that yields consistently 50-70% sporulation of two thermophilic anaerobic strains. Pea-extract media supplemented with manganese and iron were used, with close control of incubation temperature. Attempts to replace pea extract in the medium were not successful. Good progress



has been made on investigations of sporulation requirements and metabolism of these bacterial strains. The investigations also involve better methods of cleaning the spores and of determining their thermal characteristics.

Investigations of enzymes essential for spore germination are supported by a P.L. 480 grant to the National Institute of Agronomic Research in Paris. Alanine dehydrogenase and leucine dehydrogenase, which are involved in germination of B. subtilis spores, were isolated and characterized. Both have about the same molecular weight but different chemical reactions to very dilute mercuric ion concentrations. Morphological changes in spores during germination are being analyzed by electron microscopy.

2. Reducing Microbial Contamination of Processed Vegetables. Buyers of frozen foods for remanufacture have become more exacting in their specifications for levels of microbial contamination of products. We have been working cooperatively with the food preservation industry of the Pacific Northwest to obtain a clear understanding of factors that influence the microbial content of frozen vegetables. Surveys were made in plants freezing peas, beans, and cut corn. The neglect of good sanitary practices at any point in a processing line can undo the effectiveness of good practice at all other points. In one pea-freezing plant, for example, care was taken that peas passing through the blancher, quality grader, flumes, and inspection belts maintained plate counts well under the most exacting specification. In the freezing tunnel, however, which was not accessible to the cleanup crew, the bacterial count increased 10-fold between the last inspection belt and a spreader at the entrance to the freezing tunnel. This increase is enough to make the product unacceptable to buyers who specify microbial count.

In a number of investigations of commercial corn-freezing operations, high bacterial counts arose from washers, from conveyor belts which were inadequately cleaned or disinfected, from the tray spreader at the entrance of freezing tunnels, and generally from points where corn accumulated on guides, reels, or other equipment in the processing line. Accumulation of corn even at one point could contaminate the rest of the line and raise the count in the final product to more than a million viable organisms per gram. Use of in-plant chlorination, continuous rinsing of conveyor belts with chlorinated water, and split blanching operations with a second blanch as close as possible to the freezing operation, all lowered counts. Continuous attention to cleanliness of the line was found to be essential, and advice was given processors to enable them to bring microbial contamination into satisfactory control.

## E. Technology--Process and Product Development

1. Tomato Products. Tomatoes are second only to potatoes as a processed vegetable. Although there is a wide diversity of tomato products, most of them are in the form of pastes, purées, or combinations of these concentrates with spices, sugar, etc., such as catsup and pizza sauce. The consistency of these products is an important quality factor.

We have been working cooperatively with an industry committee to develop an analytical method for testing raw material to determine its potential capacity to make products of a desirable consistency. Consistency of tomato products is largely determined by the amount and quality of pectic substances. Enzyme systems exist in tomatoes that very rapidly degrade pectic substances as soon as the tissue is disrupted. Processing methods have been developed to heat tomatoes very rapidly as they are crushed in order to reduce the degrading activity of pectic enzymes. We have discovered that by crushing tomatoes in the presence of acid, the enzymes are inhibited so that we can obtain tomato juice that has not been enzymatically degraded. This observation appears quite useful for obtaining a simple reproducible method for analyzing tomatoes for processing quality. Preliminary evaluations of the method we developed were made in 1965 and, because the results were promising, more extensive commercial testing of the analytical procedure will take place in 1966.

We are also applying this new method to a process for making tomato products of controlled consistency. By adjusting the level of acidity at the time the juice is extracted, we can make a high-consistency juice, a low-consistency juice, firm tomato-juice gels, or products like catsup or sauce with intermediate consistencies. Applications have been made for two public service patents to cover these processes.

We have prepared a series of tomato juice and concentrated tomato juice products with a starting range of pH from 1.0 to 9.0, by adjusting the pH at the time of juice extraction. Analyses of pectin fractions of these juices showed that at low extraction pH (1.0 to 2.0) the total pectin content was relatively high, and the pectin had a relatively high methoxyl content and a high molecular weight. At pH levels above 5.5, a high yield of pectin was also obtained but with a low methoxyl content. Tomato pastes were prepared from juices extracted at pH 1.5, 4.2, and 6.0 and dried on the foam-mat dryer. The drying characteristics of the sample having lowest pH were superior to the others, although the salt content was high as a result of the pH adjustment and subsequent neutralization. All the samples rehydrated readily to fluid products of excellent color and consistency. We hope to find specific processes to turn out tomato products to meet specific quality and consistency requirements.

A modified rotary steam-coil low-pressure evaporator has been designed and constructed to improve quality and reduce costs of concentrating food liquids such as tomato juice and fruit preserves. We call this the WURLing evaporator. High-quality pastes of 50% solids can be produced, although few other evaporators can produce 50% tomato paste without serious quality and efficiency loss from burning of tomato solids onto the heat-transfer surfaces. A rapidly whirling coil is completely submerged in the liquid under vacuum. Both submersion of the coil and its movement in the fluid help reduce fouling. By operating under vacuum, low-temperature steam can be used in the coil, and thus permit accurate temperature control and prevent the development of hot spots. Fouling is minimized, and the quality of the product is

preserved. One large installation of the WURLing evaporator has a capacity of 4-1/2 tons of high-density tomato paste per hour. Another application of the rotary coil vacuum evaporator is in making high-quality fruit preserves; a commercial installation has been made for this purpose. The WURLing evaporator has successfully produced high-quality preserves from strawberries, orange peels, sour red cherries, apricot purée, and pineapple chunks. A minimum of fruit breakage occurred because of the low-temperature boiling and the gentle agitation from the rotating heat-transfer surface. Configurations are being considered to design a commercial-scale vacuum jam maker that could operate continuously.

2. Frozen Vegetables. Experimental packs of vegetables frozen by immersion in liquid nitrogen and by immersion in refrigerated food-grade Freon 12 were prepared and evaluated. Green snap beans, Romano beans, cob corn, cauliflower, broccoli, Brussels sprouts, carrots, and hot-house rhubarb were frozen by immersion in liquid nitrogen. Green beans, Romano beans, and cob corn were superior in texture to their conventionally frozen controls. Broccoli does not appear to be adapted to immersion freezing in liquid nitrogen, since the florets are very brittle when frozen. Immersing the broccoli heads in water before liquid-nitrogen freezing (LNF) helped to keep the spear intact but did not eliminate the shattering of the head. Shear values of LNF beans of 5 varieties were always significantly higher than those of conventionally frozen beans.

Brussels sprouts, cauliflower, cob corn, carrots and hot-house rhubarb were also frozen by immersion in Freon 12. At a temperature of about -50° F., freezing in Freon was almost as rapid as in liquid nitrogen. There was less shattering with Freon immersion than with liquid-nitrogen immersion. Brussels sprouts, cauliflower, carrot strips, and hot-house rhubarb retained their textural properties better than samples that were conventionally frozen. Liquid nitrogen appears to have a good potential because textural qualities of such vegetables as cob corn and green beans very closely resemble the fresh, whereas conventionally frozen products lose much of their textural quality on freezing.

3. Dehydrated Vegetables. Factors that affect the rehydration of dehydrated vegetables are being investigated. Celery serves as a model. Air-dried celery rehydrates to only about 1/3 of its fresh weight, but when celery is dehydrated by alcohol extraction it absorbs more than its fresh content of water. Cooked samples that had been dehydrated by extraction with various alcohols had drained weights of from 125 to 156% of their fresh weight; cooked frozen celery had 90%. Although drying by alcohol extraction is not proposed as a practical method, its use in research is beginning to lay the basis for an understanding of rehydration phenomenon, and it may lead to processing methods that will improve a serious defect in dehydrated vegetables.

4. Dry Bean Products. Development of quick-cooking Lima beans is supported in part by the California Lima Bean Advisory Board. Improvements have been



made in the product previously described. Dry beans are rehydrated in a solution of four edible salts, with rehydration accelerated by fluctuating pressure. The new product, which could be marketed in dry form closely resembling unprocessed beans, can be prepared for table use in about 30 minutes by cooking in boiling water. The cooked beans have excellent appearance and flavor, and a prolonged soaking time is avoided while cooking time is reduced. The method has been applied successfully to prepare quick-cooking products from other types of dry beans. In cooperative research with commercial processors, large samples of quick-cooking beans have been prepared for evaluation in industry.

In storage tests of dry beans, Pinto and Sanilac varieties of low moisture (8%) possessed excellent cooking qualities after 4 years in storage at 70° and 90° F. High-moisture samples (15-16%) of large Limas, Pintos and Sanilacs showed small but significant increases in cooking time after 18 months at 40° and 55° F. Several-fold increases in cooking time were observed in similar samples stored at 70° F. Grade A beans are permitted to contain as much as 18% moisture. Recent results support the earlier investigation on beans which demonstrated a loss in cookability significant at temperatures as low as 40° F. and serious at 70° F. Dry beans for prolonged storage should have moisture contents of 11% or 12% or lower.

Investigations are being carried out by contract at Michigan State University in East Lansing to scale up our process for making precooked bean, pea, and lentil powders. We hope this will speed commercialization of the process. When beans were cooked in a retort at 250° F. instead of at 212°, it was possible to dry a puree made from them on a double-drum dryer without use of additives. Modifications were made in the dryer to increase its efficiency. Optimum dryer-operating conditions were established so that two pounds of product per square foot of drum surface could be dried per hour to a final moisture content of 5%.

5. Removal of Radioactivity from Vegetables by Processing. Investigations on the effect of commercial processing operations on removal of radioactive contamination are conducted by contract with the National Canners Association's Western Laboratory in Berkeley, California. This study includes possible removal methods for external contamination from fallout and for internal contamination from sources such as radiostrontium absorbed through the soil. It was found that more strontium accumulates in the interior of the potatoes than in the peel, so that peeling potatoes grown in contaminated soil would not effectively decontaminate them. On the other hand, normal processing operations for spinach, such as washing and blanching for canning or freezing, reduced external fallout contamination by 90%. Pea seeds, primarily because of the protection afforded by the pod, contained only traces of radioactivity when harvested one hour after exposure to simulated fallout. Strontium and calcium contents of the edible portions of peas, corn, apples, peaches, and pears grown in soil fortified with stable strontium were measured. The flesh of the three fruits was much lower in strontium than were the peels, pits, or cores. Pea seeds were lower in strontium

than the pods. Thus, at least in some fruit and vegetable crops grown in soil contaminated with radioactive fallout, much less radiostrontium would be translocated into those parts used for food than into those which are normally discarded.

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AREA NO. 10. SAFFLOWER, CASTOR, AND OTHER WESTERN  
OILSEEDS UTILIZATION - FOOD

Problem. Cash crops for diversification and rotation programs need to be increased, particularly in cotton-producing areas of the western states. A crop with potential for these programs is safflower, which is emerging as an important source of edible and industrial oil as well as edible seed meal for both food and feed use. Basic information is needed on the composition of the oil and meal and, to obtain this information, adequate analytical methodology must be developed. Rapid and accurate analytical methods are also needed to control and improve the processing of oil and meal.

The high percentage of linoleic acid (essential fatty acid) in safflower oil is a feature that is leading to its rapidly expanding use as a food oil. And breeding research is producing varieties with other advantages. One new variety is very high in oleic acid, providing an oil of unusual stability against oxidation in food products and in cooking. New thin-hulled varieties give greater yields of oil and seed meal, but flavor and color problems exist in the most promising thin-hulled varieties so far developed. Utilization research is required to remove the odoriferous substances and pigments so that a light-colored bland oil is obtained.

Safflower has become a significant item of export, with 240,000 tons of safflower seed exported in calendar year 1964. This export was essentially all to hard-currency customers, so it benefits our unfavorable international trade balance.

Castor also can provide the diversification that is needed in western growing areas. But we anticipate only limited food use of it, although the oil has been used in Asia for centuries for cooking purposes.

Basic and applied research is needed to provide improved processes for and products from safflower, castor, and other western oilseeds.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, both basic and applied research are conducted on safflower and castor seed at the Division headquarters at Albany, California and under contract at Tucson, Arizona. Basic compositional studies on oilseed meals are concerned with the resolution of their water-soluble proteins and determination of their nutrient properties for food. Studies are conducted on the composition and stabilities of safflower oils. New analytical techniques also are being developed.

The Federal program of research in this area totals 2.7 scientist man-years, including contract research equivalent to approximately 0.5 scientist

man years per year. Of this total 0.5 are assigned to chemical composition and physical properties, and 2.2 to technology--process and product development.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 11 scientist man-years is devoted to Areas 10, 11, and 12.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition and Physical Properties

1. Antioxidants of Safflower Oil. Under a contract at the University of Arizona in Tucson, naturally occurring antioxidants in safflower oil are being separated, purified, and characterized. Moisture, volatiles, oil, and free fatty acid contents; peroxide value; and autoxidation induction period were determined for a series of representative safflower oils. Induction period for autoxidation was affected by light, so methods had to be developed to eliminate the effects of incident light on the reaction mixtures. Methods were also developed to isolate and measure the amounts of tocopherols present in oil. Commercial oil samples from various processing stages were examined for relative stability. In total, 28 samples of high-linoleic safflower oil have been investigated. Their autoxidation induction periods ranged from 163 hours for refined, bleached, deodorized, commercial oil to 696 hours for an oil from an experimental variety. Compositional data are incomplete, so any correlation that may exist between tocopherol content and stability of the various samples has not been established. Removal of the non-saponifiable fraction from an oil reduced its stability, but adding the non-saponifiable material from one of the stable oils to a less stable oil did not significantly increase the stability. It is proposed that natural antioxidants are destroyed or eliminated during saponification.

A high oleic safflower variety (UC-1) developed in the breeding program at the University of California at Davis was found to have an autoxidation induction period greater than 2,000 hours in the series of tests conducted at the University of Arizona.

##### B. Technology--Process and Product Development

1. Oxidative Stability of Safflower Oils. High-oleic safflower oil was evaluated for oxidative stability under high-temperature conditions, and the results were compared with those obtained with ordinary safflower oil and other vegetable oils. Crude high-oleic oil was superior to the other oils tested in that less polymerization occurred. Refined high-oleic oil was slightly inferior to a commercial blend of hydrogenated vegetable oils. Since hydrogenation costs could be avoided, however, high-oleic safflower oil, with excellent stability under conditions used in commercial food frying, can become a major cooking oil once its production is increased. The high-oleic oil is liquid at refrigerator temperatures, so it could be handled

easily from drums or bulk storage, and thus handling cost would be less for large commercial users. Several very large companies have eagerly sought information and samples of this remarkable vegetable oil.

The odor problem associated with some thin-hulled varieties continues under investigation. Hull oil obtained by solvent extraction can be further extracted with dilute base to remove color and odor. Color and odor concentrates were obtained by column chromatography from hull oil in order to study chemical composition. Data on odor- and color-causing constituents will be valuable to growers, breeders, and processors working on new promising safflower varieties.

2. Safflower Seed Meal. Partially decorticated safflower seed meals ranging from 42 to 50% protein and an experimental undecorticated meal from a new thin-hulled type of safflower seed were subjected to comprehensive chemical analysis and biological evaluation using chicks. Samples were analyzed for proximate constituents, cellulose, lignin, pentosans, water-soluble vitamins, and amino acids. Methionine and lysine were the only amino acids that are relatively deficient in the meals. When these were added and when adequate energy was supplied to compensate for the high-fiber content, safflower meals produced growth rates superior to those produced by a corn-soya ration. This indicates that safflower meals contain little if any of the deleterious components found in legume seeds, and so could provide a new and highly desirable protein source for poultry and swine. Such use will depend on economic factors involving supplementation with fat to supply the energy deficit and with lysine and methionine. Improved methods of decortication, especially as applied to the thin-hulled varieties, should enhance the potentialities for safflower meal in animal feeds and open the way for development of low-cost human foods from safflower. Removal of the disagreeable flavor in thin-hulled safflower seed from low-fiber, low-fat meal was accomplished by extracting with ethanol, acetone, or isopropanol. Protein content was increased to about 75% with a loss of about 5% of the nitrogen. Preliminary food evaluation was made by incorporating this flour in bread and in meat-substitute dishes.



AREA NO. 11. SAFFLOWER, CASTOR, AND OTHER WESTERN  
OILSEEDS UTILIZATION - FEED

Problem. Cash crops for diversification and rotation programs need to be increased, particularly in cotton-producing areas of the western states. A crop with potential for these programs is safflower, which is emerging as an important source of seed meal having great possibilities for food and feed use, in addition to its importance as a source of edible and industrial oil. Basic information is needed on the composition of meals and oil, and this in turn requires development of adequate analytical methodology. Rapid and accurate analytical methods are also needed to control and improve the processing of meals and oils for feed and other uses. Thin-hulled varieties of safflower, being developed through breeding research, will provide greater yields of seed meal and oil. We are cooperating with industry, state and federal plant breeders to develop analytical methodology needed to guide these studies.

Castor also can provide the diversification needed in western growing areas. Control of allergenic and toxic components of castor meal would make available a high-protein product for feed and food uses. We import much of the castor oil required for industrial use, but better utilization of domestic castor seed oil meal would increase the total value of the domestic crop and be an incentive to increase the acreage and reduce our dependence on imports. Safflower, on the other hand, has become a significant item of export with 240,000 tons of safflower seed exported in calendar year 1964. This export was essentially all to hard-currency customers, so the export of safflower and the decreased import of castor would both benefit our unfavorable international trade balance. Basic and applied research is needed to provide improved processes for the products from safflower, castor and other western oilseeds.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, research on developing new and improved feeds from safflower, castor, and other western oilseeds is conducted at Albany, California. The Federal program of research in this area is equivalent to about 1.6 scientist man-years per year.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 11 scientist man-years is devoted to Areas 10, 11, and 12.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Technology--Process and Product Development

1. Improved Feeds from Oilseed Meals. Information and processes are being developed to produce a low-fiber safflower meal and to determine its feed

value. Decortication and hull removal prior to oil extraction from safflower seeds was used to prepare 40 kilograms of meal for evaluation in animal rations. Initial studies of commercial and laboratory-processed meals indicate that commercial operations have reduced the solubility of protein. Chick-feeding tests show that safflower meal contains little if any deleterious components. (See paragraph 10-B-2.)

Primary utilization problems in feeds, then, revolve around the low metabolizable energy of safflower meals. The hulls are relatively indigestible, and the methionine and lysine content of safflower protein is low. When meals are supplemented with an energy source and with lysine and methionine, growth rates obtained in broiler chicks were outstanding. When low-fiber meals can be produced, perhaps by decortication, larger markets can be expected for the meal in poultry and swine rations. Potential markets for improved safflower meal exist in the United States and abroad.

Castor meal can be deallergenated with lime, under the following process conditions: temperature, at least 120° C., preferably 140° C.; time, 1 hour or more; liquid-to-solids ratio, 3; lime concentration, at least 8% by weight. The high lime content limits the usefulness of the product, hence further studies are being made with sodium hydroxide, alone and in combination with lime. At present, the lime treatment can be considered as only a stopgap measure; cost reduction is needed. New work on a high-pressure steam process for castor meal deallergenation is promising. It is evident that steam pressures in excess of 80 lbs. per sq. in. will be required to avoid long processing times.

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AREA NO. 12. SAFFLOWER, CASTOR, AND OTHER WESTERN  
OILSEEDS UTILIZATION - INDUSTRIAL PRODUCTS

Problem. Cash crops for diversification and rotation programs need to be increased, particularly in the cotton-producing areas of the western states. A crop with potential for these programs is safflower, which is emerging as an important source of industrial and edible oil, as well as of seed meal that may find uses in foods and feeds. Basic information is needed on the composition of the oil, and this requires development of adequate analytical methodology. Rapid and accurate analytical methods are also needed to control and improve the processing of oil for industrial applications. Safflower oil is particularly valued because of its non-yellowing qualities when used in surface coatings. Breeding research has yielded varieties of safflower with wide variation in fatty acid contents, pointing to the opportunity of growing specific crops for specific applications.

Castor also can provide the diversification that is needed in western growing areas. Utilization research is pointing the way to improved products such as lubricants and foamed polyurethane plastics. Domestic production of castor is so limited that much of our United States requirement must be imported, and large stocks are held by the government as a strategic reserve. Control of allergenic and toxic components of castor meal would make available a high-protein product for feed and food uses. Better utilization of domestic castor seed oil meal could increase the total value of the domestic crop and be an incentive to increase the acreage and thus reduce our dependence on imports. Safflower, on the other hand, has become a significant item of export with 240,000 tons of safflower seed exported in calendar year 1964. This export was essentially all to hard-currency customers, so the export of safflower and the decreased import of castor would both benefit our unfavorable international trade balance. Basic and applied research is needed to provide improved processes for and products from safflower, castor and other western oilseeds.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, both basic and applied research are conducted on castor and safflower seed at the Division headquarters at Albany, California; under contract in Tucson, Arizona, Minneapolis, Minnesota, and Fargo, North Dakota; and by P.L. 480 grant funds in India. Studies are conducted on the composition of castor and safflower oils and on new products therefrom. New analytical and preparative techniques are developed.

The Federal program of research in this area totals 6.3 scientist man-years, including contract research equivalent to approximately 1.5 scientist man-years per year. Of this total 1.9 are assigned to chemical composition,



physical properties and structure; 2.1 to chemical and physical investigations to improve products; and 2.3 to technology--process and product development. In addition, three grants are sponsored under P.L. 480.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 11 scientist man-years is devoted to Areas 10, 11, and 12.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition, Physical Properties and Structure

1. Oil and Oilseed Components. The absolute configurations of dimorphecolic, lesquerolic, and densipolic acids, derivatives of Dimorphotheca and Lesquerella species, were determined. Highly purified samples of the methyl esters of these long-chain unsaturated acids and their saturated derivatives were examined by spectropolarimetry. A D-configuration was established for all the derivatives. Confirmation of configurational assignments was obtained by using the reduced form of the hydroxy acids, because we have observed that unsaturated acids of identical configuration give different data. The absolute configuration of lesquerolic acid was unequivocally established by its synthesis (in six steps) from ricinoleic acid of known configuration.

The far ultraviolet spectra of a series of cis and trans, mono- and poly-substituted unsaturated fatty acid esters were examined spectrophotometrically. Data obtained showed some inconsistencies with earlier results. Spectral data are useful to establish existence of such acids in unknown mixtures and thereby to characterize chemical reactions that occur in the processing of oils and synthesis of useful industrial compounds.

Analysis of current commercial safflower seed types shows that they are composed of about 40% hull, 37% oil, and 23% meal. Experimental varieties exist with hull percentages ranging from 59 to 18 and inversely varying oil and meal percentages. In safflowers of high linoleic acid content, fatty acid composition is quite constant. In genetic studies selections with equal oleic and linoleic acid contents, with high oleic and low linoleic acid content, and with relatively high stearic acid contents are being obtained. The high degree of genetic control of fatty acid content offers a great potential for improving safflower varieties. Varieties will probably be bred for a specific fatty acid content depending upon the industrial products they are destined for.

##### B. Chemical and Physical Investigations to Improve Products

1. Oil Derivatives. Improved methods reported previously were used to prepare large quantities of methyl 12-ketostearate. High conversion and yield of pure product were obtained with low levels of commercial copper chromite catalyst. By using distilled commercial methyl 12-hydroxystearate, 96% conversion was obtained. A sample of pure N,N-dimethyl 12-acryloxystearamide



was provided for our contractor at the University of Arizona for polymerization studies. Methyl 12-ketostearate and 12-ketostearamide were sent on request to Armour Industrial Chemical Company for evaluation. Methyl 12-ketostearate was sent on request to the Baker Castor Oil Company.

Improved methods of alkali fusion have been developed and new phosphorus derivatives prepared for evaluation. In contract research at the University of Arizona good progress has been made on polymerization and evaluation studies on monomers derived from oilseeds. Evaluation of such new polymers and copolymers for possible use in rubber modification, coating applications, and adhesive compounds should provide more industrial outlets for oilseeds.

Keto derivatives and other potentially useful compounds, including polymerizable monomers, were prepared. Improved methods were developed for preparing omega-hydroxy acids by alkaline fusion. The starting material is a hydroxy unsaturated acid, such as ricinoleic acid from castor or lesquerolic acid from Lesquerella species. Transformation of hydroxy fatty acid esters to keto fatty acid esters were monitored successfully by using gas liquid chromatography. This was made possible by converting the hydroxy compounds to their trifluoroacetyl or trimethylsilyl derivatives.

Investigations of the preparation of polymerizable monomers from castor oil hydroxy fatty acids are supported by P.L. 480 grant funds to the Regional Research Laboratory in Hyderabad, India. Methods for preparing acryloxy and methacryloxy monomers from castor oil hydroxy fatty acids were considerably improved to get higher yields and better quality products. Preparation, purification, and characterization of six monomers obtainable from castor oil have been described. A considerable number of monomers were sent to the Western Utilization Research and Development Division for examination and evaluation in polymerization systems.

Another P.L. 480 research grant to the same institution is used for investigating selected hydroxylated derivatives of linseed and safflower oils. The project is under the joint supervision of the Western and Northern Divisions. Three chemical routes have been followed for introducing monohydroxy functions into unsaturated oils. To sulfate safflower oil, 86% sulfuric acid was used. The subsequent hydrolysis of sulfate to hydroxyl groups was accomplished by using acidified barium chloride solution. Only about half the unsaturation lost was accountable as new hydroxyl groups. Partially epoxidized unsaturated esters or oils were converted to the corresponding monohydroxy products by hydrogenation in ethanol medium at a high pressure. Safflower oil was peroxidized by bubbling air at 0° C. for 40 hours under ultraviolet irradiation. Subsequent reduction of the hydroperoxide to hydroxy groups was easily achieved by a number of procedures. Catalytic hydrogenation yielded the highest hydroxyl value. Build-up of hydroxyl by peroxidation to a low value, reduction to hydroxyl, and further peroxidation led to thick dark unsatisfactory products.

### C. Technology--Process and Product Development

1. Castor-Based Products. New castor oil derivatives that may be useful in flame-resistant foams were made by incorporating chlorine or bromine. Castor oil-based components with improved storage stability are being developed, with special attention being given to flame resistance which is especially needed in structural uses of rigid urethane foams. A series of rigid foams was prepared from homopolymers and copolymers of vinyl 12-hydroxystearates. Properties of these foams were generally inferior to those of similar foams prepared from castor oil or hydrogenated castor oil. The resistance of several castor oil-based urethane foams to attack by fungi and other micro-organisms is under investigation. No loss of strength or visible signs of attack were evident in castor oil-based urethane foams after 6 months' burial in moist earth.

An investigation of continuous production and evaluation of castor-based urethane foams is being conducted by contract with the Archer-Daniels-Midland Company. Castor formulations provided by the Western Utilization Research and Development Division were used for preliminary evaluations. Flame retardants, synthetic polyols to blend with castor oil, different isocyanates in the urethane formulation, silicone surfactants, and castor oils of different grades are included in these tests. Two commercially available isocyanates were used to prepare acceptable foams from castor-based polyols with equivalent weights as high as 120. Prepolymer systems or crude isocyanates require castor-based polyols with equivalent weights in the 50-100 range for adequate foams. The initial screening results conducted under this contract corroborate much of the batch-operation laboratory work we have done.

Highly efficient methods have been developed for converting low-cost hydrogenated castor oil esters to ketostearate esters which have unique physical and chemical properties that are useful in permanent-type automotive lubricants and mold-release compounds. Inexpensive catalysts rapidly convert the castor esters to ketostearates--processing costs are estimated at only a few cents a pound. A yearly market for several million pounds of ketostearates in lubricant application is anticipated, and now that ketostearates can be prepared inexpensively, exploratory investigations can be launched to find new industrial uses for this type of compound.

Castor-based and polyether-based rigid urethane foams have been compared as to the effect of aging on their thermal conductivity. Uncut samples of both types of foam (representing foam-in-place applications) maintained equivalent low thermal conductivities throughout test periods of 6 to 12 months. Common practice, however, is to cut samples for testing. With such samples, thermal conductivities increased at different rates. The initial increase in thermal conductivity of castor oil-based foams was greater than in polyether-based foams, because the rate of diffusion of air into the foam cells was higher. However, tests with uncut samples are the more realistic, so we conclude that castor-based foams are not inferior for many of the most important uses for polyurethanes.

By a method developed here, controlled alkali-fusion of castor oil has yielded predominantly omega-hydroxy acid rather than sebacic acid. This method is under investigation by two large producers of fatty derivatives. They undoubtedly plan scale-ups to produce this unique chemical for large-volume use in plastics and biodegradable detergents, and in the manufacture of other chemicals.

2. Lesquerella and Dimorphotheca. Evaluation of Lesquerella and Dimorphotheca as possible replacement crops has been discontinued. However, contract research at North Dakota State University on surface coatings from hydroxy unsaturated oils continued into this reporting period and studies of the properties of dehydrated Lesquerella and Dimorphotheca oil have been completed. Fatty acids of Dimorphotheca oil contain up to 60% of 9-hydroxy-trans-10-trans-12-octadecadienoic acid. The oil itself dries poorly in the presence of conventional dryers. Addition of nonconjugated cis triglycerides, for example 5% of linseed oil, bring about a marked improvement in the drying properties of this oil. The hydroxyl group of dimorphecolic acid was used advantageously in preparation of film-forming compositions. Coatings with excellent properties have been prepared by reacting the oil with several mono- and poly-isocyanates. The combination of the oil modified with rosin esters by means of the poly-functional isocyanates was studied. Both mono- and poly-isocyanates and modifying resins had a significant effect on the physical properties of films.



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### AREA NO. 13. POULTRY UTILIZATION - FOOD

Problem. In spite of a 60% increase in production over the past decade, gross farm income from poultry meat has increased only from \$1.4 to \$1.5 billion. The poultry meat industry operates on very narrow profit margins. In order to keep up with developments in the modern food industry, poultry must be converted into a wide variety of products having high quality and convenience, at costs attractive to consumers and remunerative to the poultry grower. More information on the properties and processing of poultry is needed to enable us to better utilize poultry in a variety of forms attractive to consumers. Increased utilization of poultry would serve toward eliminating our feed grain surplus, increase returns to farmers, and provide better products for American consumers.

Although poultry is an efficient converter of feed to meat, more grain is used by poultry per calorie of food produced than by any other commercial animal because a high percentage of the poultry diet is grain and because poultry meat contains exceedingly little fat. One-fourth of all grain fed to animals is used for poultry and egg production. Hence, increased consumption of poultry products would be an effective means of increasing markets for surplus grain. Also, the efficiency of feed utilization by poultry makes prices for poultry meat low and, thus, within reach of more consumers, with the result that the nutrition of consumers who now have diets low in animal protein is improved.

The consumption of poultry has steadily increased from a 1947-1949 average of 22 lbs. per capita to 39 lbs. in 1963. This important increase has involved price, quality of product, availability, and disposable income. Because of the current low profit margin, increasing consumption by lowering farm prices is impractical. Increased demand for and consumption of poultry will require higher quality, more convenient products and a greater variety to meet the desires of the modern consumer. In addition to greater returns from increased demand, a greater profit margin for the farmer can, of course, come from greater efficiencies in processing.

The trend toward convenience foods and further processing has led primarily to precooked poultry products which are generally less stable, more subject to warmed-over flavors, and more likely to provide texture problems than are uncooked items. With the expansion of operation and the emphasis on continuous, more efficient processing, need has arisen for better procedures for feather removal, chilling, tenderizing, freezing, deboning, and commercial cooking. Lowering the cost and improving the quality of products that can be stored at ambient temperatures, such as canned, dried, cured, and irradiated products, offer potential for poultry utilization in domestic and export markets. As a foundation for applied studies, further knowledge is needed on the chemical nature of flavor and flavor changes during processing and storage, on tenderness development, and on proteins, lipids, and other components.



## USDA AND COOPERATIVE PROGRAM

Basic and applied research on poultry meat and poultry meat products are conducted at the Division headquarters at Albany, California, and by contract in Madison, Wisconsin, and Berkeley, California. Fundamental studies on poultry flavor are concerned with the identification of flavor precursors in poultry meat and the isolation and identification of volatile flavor components developed during cooking. The chemistry of muscle protein and post mortem chemical changes are investigated relative to tenderness and other quality characteristics of poultry. The basic physiology of the feather-release mechanism in fowls is studied. Applied research is conducted on the stability of cold-tolerant organisms; special problems of flavor, texture and stability of precooked frozen foods; and processing factors that influence tenderness of poultry meat.

The Federal program of research in this area totals 11.2 scientist man-years, including contract research equivalent to approximately 1.4 scientist man-years per year. Of this number, 4.1 are assigned to Flavor; 4.3 to Color, Texture, and Other Quality Factors; and 2.8 to Technology--Process and Product Development.

### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 17 scientist man-years is devoted to this area of research.

### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

#### A. Chemical Composition and Physical Properties

1. Poultry Meat Components and Properties. Contract research at the American Foundation for Biological Research, Madison, Wisconsin, was conducted to obtain a better understanding of the ultrastructure of poultry muscle tissue and how this structure is modified by variations in aging, freezing, thawing, cooking, and related food-processing steps. Electron microscopic examinations revealed several distinct graded patterns of change in muscle tissue as it progressed from the pre-rigor to the post-rigor state. Freezing and thawing or heating had marked effects on the structure of pre-rigor muscle, but less effect on the post-rigor muscle. As expected, heating effects dominated the picture. Combined effects of successive freezing and heating or heating and freezing on pre-rigor and post-rigor muscle were largely determined by the severity of the first condition applied. These studies constitute the first essential step towards applying data on ultrastructure to the development of an explanation and a control of quality losses in poultry meat subjected to various processing operations. To follow the promising leads of this research, another contract has been negotiated with the American Foundation for Biological Research.

In research that is supported in part by the U.S. Public Health Service Career Award Program, enzymes from chicken and turkey pancreas are being

compared with their counterparts from other animal sources. Chymotrypsin isolated from chicken differed from mammalian chymotrypsin both in physical and in enzymic properties. Trypsin isolated from turkey, on the other hand, did not differ in enzymic properties from mammalian trypsin, although their physical properties differed. The yields and properties of the enzymes suggest that they perform the same role in intestinal digestion in birds that they do in mammals.

Fluoride content in the back and neck parts of commercial broilers was evaluated as a basis for decisions on the relative suitability of the hard-to-market backs and necks as a finely ground meat and bone emulsion for use in food products. Fluorine concentration in complete backs and necks (meat, skin and bone) was 20 times that in poultry meat (wet basis). About 1 mg. of fluorine would be expected from an average (56-gm.) portion of backs and necks, a value much lower than some previously reported. Cooperative studies in which broilers were fed with a wide range of phosphorus supplements indicated that the source of phosphorus in the feed is a major factor controlling fluorine content of the carcass. At present, with due consideration of the above data, the Food and Drug Administration questions the wisdom of using bone-containing poultry meat products in foods for human consumption.

## B. Flavor

1. Poultry Aroma. Basic and applied research are being conducted on the factors that influence flavor of cooked poultry meat. Gas chromatography and other laboratory techniques have separated more than 200 volatile components from cooking chicken. Sensory tests are being used to evaluate fractions of the chicken aroma which were separated by absorbing (on powders or in solutions of metal salts) the volatile materials of freshly formed cooked-chicken aroma carried in a nitrogen gas stream. Some of the aroma constituents are not absorbed, and these were smelled by a trained panel and then characterized or identified by subjective evaluation prior to further chemical characterization. By means of these techniques, the important role of hydrogen sulfide in cooked-chicken flavor has been further revealed. However, other aromatic materials blend with the hydrogen sulfide or mask it to produce the characteristic aroma of cooked chicken.

Taste panel tests were used to demonstrate that the fat of chicken is not a direct precursor of basic chicken flavor. Fat separated from uncooked chicken was odorless and, upon cooking, it did not develop an odor or flavor typical of cooked chicken. However, the fat that exists in cooked chicken appears to serve as a solvent and a repository for the cooked-chicken aroma that develops in the nonfat components.

## C. Color, Texture, and Other Quality Factors

1. Texture of Poultry Meat. Basic biochemical relationships between changes in poultry muscle after slaughter and the ultimate tenderness of cooked meat have been under investigation for the past 10 years, with particular attention

to the fate of certain chemicals (adenosine triphosphate (ATP), phosphoryl-creatine, glycogen, and lactic acid) involved in anaerobic glycolysis during the post-mortem period when tenderness changes occur. Treatments that accelerated the disappearance of ATP and glycogen also accelerated the onset of rigor mortis and induced a toughness that was only partially reversed by prolonged aging. Treatments included severe mechanical feather picking, freezing and thawing, holding at elevated temperature, cutting the muscle, electron irradiation, and exhaustive electrical stimulation. Breakdown of glycogen can be minimized by injecting the birds with epinephrine or sodium iodoacetate prior to slaughter, or by rapid cooking of the meat immediately after slaughter. Although these treatments accelerate rigor mortis, the resulting meat is tender without aging. Evidently, therefore, acceleration of post-mortem glycolysis, rather than acceleration of rigor mortis, induces toughness in broilers.

Economic pressure to speed up processing and eliminate extended costly aging periods has raised anew questions on required aging times for complete tenderization. Research findings from this and other laboratories indicate that small turkeys and chickens vary greatly from bird to bird in their tenderness if they are not adequately aged; that at least 8 hours of post-mortem aging at temperatures above freezing is required for chickens or small turkeys to become uniformly tender; that large turkeys (20 lb. males) are adequately tender with only 1-2 hours of post-mortem aging, although holding for an additional 18 hours significantly increases their tenderness; that rapid chilling does not alter the rate of tenderization; and that agitation and manipulation of turkey carcasses during killing do not reduce aging requirements.

In addition to sensory evaluations of tenderness, objective tests of tenderness have been made by means of the Warner-Bratzler and LEE-Kramer shear force instruments. These objective methods measure the force required to shear meat in a direction perpendicular to the fiber, and they generally correlate well with relative toughness. A simple mechanical method has now been devised to measure relative cohesiveness--the effort required to separate the fibers of a meat sample. Such data may prove useful in measuring toughness and tenderness of meat.

#### D. Technology--Process and Product Development

1. Freeze-drying. A rotating vacuum-freeze dryer has been designed for the continuous freeze-drying of various foods. The equipment was completely satisfactory for drying diced cooked chicken and turkey meat, vegetables, and fruits that do not tend to stick, but the problem of some products sticking to the heat-transfer surfaces has not been overcome. Commercial adoption of such a design probably depends upon development of a configuration suitable for freeze-drying products such as cut fruit pieces--especially strawberries.



Contract research at the University of California in Berkeley is underway to develop engineering information on rate-controlling factors in freeze-drying and methods of programming heat input and determining drying end points. Thermal conductivity of freeze-dried poultry meat was determined and found to be extremely low. It is influenced materially by the moisture content (conductivity increases with increasing moisture content), and it increases very slightly with increasing pressure in the dryer. Heat conduction across the grain of the meat is much poorer than along the grain. Initial data on rate-controlling processes in freeze-dryers were compared with values computed on the basis of all the water in the meat, which forms a retreating ice front as drying progresses. The mathematical model is adequate until 80% of the water has been removed from the meat, then the removal of adsorbed water slows drying and upsets the mathematical model.

The major defects in freeze-dried poultry meat are toughness and dryness. Since commercial freeze-dried poultry meat is almost always prepared from 1- to 2-year old birds, in which there is considerable connective tissue which influences the texture of the product, attention is now being directed toward this connective tissue. The influence that aging of meat may have on the tests designed to measure connective tissue content of raw muscle has been investigated. No significant changes were observed in tissues aged for 24 hours. The difference in quality between reconstituted freeze-dried poultry meat and fresh meat may be related to differences in the way water is held within and between the structural elements of the muscle. Several proteins have been isolated from muscle fiber and partially purified for study of the effects of freezing and drying conditions on their water-holding characteristics, solubility, and other properties.

Taste panels have compared tenderness of turkey samples that had been either partially freeze-dried or completely freeze-dried before cooking. The completely freeze-dried samples were tougher. These data indicate that an appreciable part of the quality loss due to freeze-drying, as in most drying processes, occurs in the later drying stages, after much of the moisture has already been removed.

PUBLICATIONS - USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

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#### AREA NO. 14. EGG UTILIZATION - FOOD

Problem. Between 1955 and 1965, gross farm income from eggs decreased from \$1.96 billion to \$1.81 billion, in spite of an increase in total production from 60 billion eggs to 66 billion in that time. This increase, however, represents a decline in per capita consumption from 371 to 308. Because the demand for table eggs is inelastic, increased utilization of eggs must come mainly from products that emphasize quality and convenience. Adequate knowledge is lacking of the properties, processing characteristics, and new-product potentials of eggs to develop new markets. Present outlets for the 10% of egg production that is frozen or dried include the baking, confectionery, salad dressing, noodle, and baby food trades. Modified and new products emphasizing quality and convenience are needed to increase acceptance of egg products by these industries and to compete successfully with egg substitutes. Improved egg-containing products would benefit the producer in three ways: by providing an increasingly useful buffer for stabilizing egg prices; by providing additional uses and outlets for eggs; and by providing more remunerative outlets for wholesome eggs that are unsuitable for table use because of appearance or handling characteristics.

Further basic research on egg composition and components is essential to reach an understanding of physical and chemical changes accompanying processing, storage and use of eggs. The basic information will be used to devise better processes and products, including new household and institutional products. Studies must encompass a full appraisal of physical, chemical, and microbiological problems peculiar to the formulated products.

#### USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic and applied research is conducted at the Division headquarters at Albany, California; by contracts and grants in Ames, Iowa, Ithaca, New York, and Davis, California; and by grant funds under P.L. 480 in France, Australia and India. Fundamental research is conducted on egg proteins and their relations to the functional properties and quality in eggs, on egg lipids and their role in off-flavor development in yolk solids, on the mechanism of bacterial penetration and survival in eggs, and on the bactericidal, antiseptic, anti-inflammatory, and food preservative properties of lysozymes and other components from eggs. Applied research is conducted on the stabilization of yolk-containing solids to increase the usefulness of eggs in dry mixes and other convenience foods, on new and improved drying procedures to make dried egg fractions and products more readily and more completely dispersible, on various methods of controlling Salmonella in eggs, and on factors in the handling of shell eggs that affect egg product quality and cost.

The Federal program of research in this area totals 13.1 scientist man-years, including contracts and grants equivalent to approximately 2.2 scientist man-years per year. Of this number 3.6 are assigned to chemical composition and physical properties; 2.9 to microbiology and toxicology; and 6.6 to technology--process and product development. In addition, three research grants are supported by P.L. 480 funds.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 14 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition and Physical Properties

1. Composition and Methodology. Lysozymes are proteins with biological properties including enzymic and antibiotic activity. Basic investigations of lysozymes from eggs and other animal sources have been conducted at the University of Paris, France, supported by a P.L. 480 grant. In this program, lysozyme from chicken egg whites was studied to determine the relationship between chemical structure and biological activity, and compared with lysozymes from other sources. The egg white lysozyme was broken down by an amino peptidase enzyme, to yield a number of derivatives, the principal one of which remained as active as the native lysozyme.

Research supported by P.L. 480 funds at the Indian Institute of Science in Bangalore is designed to determine changes in physical chemical properties in proteins of hen egg yolks that occur on freezing and thawing, namely, the gelation of egg yolk. Egg yolk was treated with formic acid to partially remove lipids, then the residue was dialyzed to remove the formic acid solution. On dialysis, one part of the protein gelled in the dialysis bag, while the other part remained in solution. The gelled and soluble proteins were separated by centrifuging. They contained about 20 to 24% lipid, mainly lecithin, but also neutral lipids and phosphatidyl ethanolamine. The gelled protein fraction gels in 50% aqueous formic acid, whereas the soluble protein fraction forms only a viscous solution with 50% formic acid. Both fractions gel instantly in a solution of 50% dimethyl sulfoxide and 50% formic acid. The chemical treatments used may have denatured the egg yolk proteins so they no longer are closely related to the native protein. Nevertheless, the information will be helpful in developing a thorough understanding of the elusive mechanism(s) involved in the marked change in physical properties of yolk that occurs on freezing.

A basic study of ovalbumin in eggs is being conducted at the Commonwealth Scientific and Industrial Research Organization, Ryde, New South Wales, Australia, supported by P.L. 480 grant funds. Ovalbumin, the main protein of egg white, occurs in two forms which differ greatly in resistance to denaturation but are otherwise very similar. The more stable form, S-ovalbumin, is formed from the original ovalbumin during the storage of



whole eggs and can also be produced from isolated ovalbumin by storage in slightly alkaline solution. It was postulated that the change involved a change in the disulfide bonding. Analyses of the chemical structure of ovalbumin and S-ovalbumin indicate that the differences in stability are not associated with differences in the intramolecular disulfide bonding that holds the untreated proteins in the coiled or folded form. Thus far only the fragments containing disulfide bonds have been examined. In future work, other parts of the ovalbumin and S-ovalbumin molecules will be scrutinized.

In-house research coordinated with the P.L. 480 projects involves isolation of individual egg proteins and characterization of their physical and chemical properties, particularly properties that might be related to the contributions of individual proteins to useful properties of egg products. Heat stabilities of different egg white proteins have been evaluated relative to the thermal destruction of Salmonella contaminants. Lysozyme is rapidly destroyed when heated in egg white at pH 9. Ovalbumin is converted to the more heat-stable S-ovalbumin. The destruction of lysozyme is the result of a reaction with ovalbumin, even though ovalbumin by itself is not appreciably denatured under these heating conditions. When egg white at pH 9 is heated to temperatures slightly above 57° C., the viscosity increases and foaming power decreases. These changes are not yet understood in terms of changes in individual egg white components, however they undoubtedly involve the lysozyme-ovalbumin reaction and other proteins. Improvements in the properties of egg products, including the performance of pasteurized eggs, depends on our understanding and control of such changes in individual proteins.

Basic studies are underway to characterize two globulins of egg white about which there is very little information. One globulin, as yet unidentified except by differentiation in electrophoretic equipment, has been fractionated, and evaluation of its molecular weight is underway.

## B. Microbiology and Toxicology

1. Spoilage and Pathogenic Microorganisms. The microbiology of spoilage and control of pathogens in egg products continue to dominate the interests in egg utilization research (see also C. Technology--Process and Product Development). The most prevalent type of pathogen that contaminates egg products is Salmonella. An egg white pasteurization method has been developed that allows use of temperatures up to 62° C. without coagulation of egg white protein. A temperature of 60-62° C. for 3-1/2 min. is adequate to destroy ordinary Salmonella contaminants in whole egg and neutralized egg white. One strain of Salmonella, however, Salmonella senftenberg 775W, which was isolated in another laboratory in 1946, is 10 to 20 times as heat resistant as other senftenbergs or other Salmonella species. Although re-isolation from food of this heat-resistant strain has never again been reported, the strain has been maintained in laboratories and its heat resistance studied extensively. Major concern exists about the variable heat resistance of the 800 to 900 different sero-types of Salmonella. Several hundred have been tested, and only one type, other than the S. senftenberg mentioned, showed

somewhat higher than normal heat resistance, but it was much lower than the S. senftenberg 775W. Danger from this one strain is minor to negligible. One report in the literature indicates that Salmonella heated in ground chicken muscle has greater heat resistance than does the same type heated in a broth suspension. Our studies with Salmonella of normal heat resistance and with the highly heat-resistant S. senftenberg 775W show that heat resistance of Salmonella in ground chicken muscle is similar to its heat resistance in other media.

A research grant was made to Cornell University to study the selenium metabolism of Salmonella, because it is known that these microorganisms grow more readily than other microorganisms on selenite media. The selenite cysteine medium, which is selective for Salmonella, loses its selectivity if glucose or 10% egg white is added. These findings may explain the variability sometimes encountered in assaying for Salmonella at very low levels of contamination, where the large samples required introduce significant amounts of glucose and egg white to the medium. Continuation of this work is expected to lead to more reliable methods.

Contract research to determine factors contributing to Salmonella contamination was concluded at Iowa State University. Direct inoculation of hens' ovaries yielded only 10 out of 38 eggs with Salmonella contamination, and that at a very low level. When hens were fed the inoculum, they did not produce eggs containing Salmonella, but oral inoculation did lead to shedding of viable Salmonella in the feces for up to 17 days after exposure. Shells of some eggs laid by such hens were contaminated with Salmonella and led to subsequent microbial contamination. Such data emphasize the importance of providing Salmonella-free feed to laying hens, since the major source of contamination of eggs is from extraneous sources.

Salmonella contaminating the shells of eggs normally does not penetrate to the egg contents, and generally the organisms decrease in number in the shell membrane system if the eggs are kept dry. The bacteria do multiply on the shells and in the membrane system in an atmosphere at 15 to 40° C. and 100% relative humidity. The numbers on the shells of washed eggs decrease by about 99% during the first 2 hours of drying. In breaking plants, a primary source of contamination of liquid egg is the flora on the egg shell, and the incidence of Salmonella contamination is increased when the general bacteriological quality of the shell eggs used for production of liquid egg is poor. Sanitizing agents and ethylene oxide treatments destroy Salmonella on the shells and in the shell membrane system. Careful plant sanitation and use of high-quality eggs can reduce the contamination of liquid egg but, in addition, pasteurization is necessary for the complete elimination of Salmonella from broken out eggs.

### C. Technology--Process and Product Development

1. Pasteurization of Egg Products. Egg products that contain Salmonella are considered to be adulterated and, therefore, cannot be sold. Department



of Agriculture poultry inspection regulations went into effect in June 1966 requiring freedom from Salmonella in egg products. The regulations specify pasteurization conditions that will assure freedom from Salmonella. Egg products so treated under continuous Department inspection need not be further tested for Salmonella contamination. Egg products prepared without the specified processes, or produced in the absence of Department continuous inspection, must be tested by a suitable method for absence of Salmonella before they can enter interstate trade channels. One of the methods specified was developed at the Western Utilization Research and Development Division. Aluminum salts are added to the liquid white and the pH reduced to stabilize the components against heat, then the egg white can be pasteurized at temperatures high enough to control Salmonella. A public service patent, No. 3,251,694, was issued May 17, 1966 and 48 commercial egg processors have received licenses to operate under the patent.

Although most properties of egg white are unchanged by the new pasteurization process, whipping time of the egg whites is increased. Basic and applied research on this damage is being conducted. A new whipping aid, triethylphosphate, has been found that is very effective in reducing the whipping time; application for a public service patent on this use has been filed.

Effect of pasteurizing temperatures on fluidity of various egg products is being determined. At suitable pasteurizing temperatures, just over 60° C., egg whites are only 2 to 3 times more viscous than water, and whole egg only 6 to 8 times. Yolk and sugared yolk are 200 to 400 times more viscous than water, and salted yolk is so viscous that its flow characteristics are very difficult to measure. A high level of viscosity will reduce pasteurizing efficiency, because flow becomes laminar, and the minimum residence time in holding tubes will be shorter than the calculated average. Attempts are being made to find an enzyme test that can be used to determine when egg products have received an adequate pasteurization treatment. The phosphatase test used for milk and milk products is not suitable because this enzyme is not inactivated by present pasteurization procedures.

Contract research is being conducted at the University of California at Davis on potential methods for eliminating Salmonella from egg products by chemical treatment. Hydrogen peroxide in concentrations from 0.3% to 3.0% is being tested over a range of acidity, without heating and with heating at 47° C. (117° F.). Preliminary tests show that the peroxide can adversely affect the conalbumin and perhaps the ovalbumin also. Peroxide caused marked changes in the lipid fraction of the yolk. It appears unlikely that peroxide could be used in a process for pasteurizing yolk-containing products, although the results obtained do not invalidate the heat plus peroxide method now being used to pasteurize egg whites.

Contract research has been initiated at Iowa State University in Ames to evaluate chemical, physical, and enzymic means of altering the functional properties of egg white to develop improved or new egg white products. Initial work involved the selection of methods of analysis and evaluation. Limited observations on egg white treated with the protein-modifying agent, potassium peroxydisulfate, show that readily detected chemical modification can occur without damage to functional properties. These results encourage further studies.



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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in	
			Summary of Progress	Area & Subheading
W1 2-27 (Rev.)	Wheat feed products	Albany, Calif.	Yes	2-A-1 2-A-2 2-B-2 1-B-1
W1 2-29 (Rev.)	Bread flavors	Albany, Calif.	Yes	1-A-1
W1 2-41 <sup>1</sup>	Wheat endosperm constituents	Albany, Calif.	Yes	1-E-2
W1 2-43 (Rev.)	Gluten foods	Albany, Calif.	Yes	1-C-2
W1 2-44	Protein interactions	Albany, Calif.	Yes	1-D-1
W1 2-47 (C) <sup>1</sup>	Elimination of microbial contaminants of wheat flour	Chicago, Ill.	Yes	1-A-1
W1 2-48 (C)	Identification of wheat proteins by radiotracer techniques	Pullman, Wash.	Yes	1-A-1
W1 2-49 (C)	Protein and lipid composition of spring and winter wheat	Manhattan, Kansas	Yes	1-A-2 1-C-2
W1 2-50	Mechanism of flour maturation	Albany, Calif.	Yes	1-C-2
W1 2-51	Compositional factors of wheat relative to continuous-mix processes	Albany, Calif.	Yes	1-C-1
W1 2-52 (C)	Wheat bran and aleurone pigments	Corvallis, Oregon	Yes	1-E-2 2-B-1
W1 2-53	Protein-rich fractions from mill run	Albany, Calif.	Yes	1-E-1 1-E-4
W1 2-54	Light-colored bulgur for specific markets	Albany, Calif.	Yes	1-A-2
W1 2-55 (Gr.)	Oxidation-reduction enzymes	Madison, Wisconsin	Yes	1-C-3
W1 2-56 (C)	Rheological study of doughs	Menlo Park, Calif.	Yes	1-E-3
W1 2-57 (C)	Carrying capacity of HRW wheats	Manhattan, Kansas	Yes	1-E-4
W1 2-58 (C)	Protein changes during malting	St. Paul, Minnesota	Yes	No <sup>2</sup>
W1 2-59 (C)	Macromolecular structures of flour	Chicago, Ill.		3-A-1 3-B-1
W1 2-63	Rice product developments	Albany, Calif.	Yes	4-A-1 4-B-1 4-B-2
W1 3-16 (Rev.2)	Improved forage feed products	Albany, Calif.	Yes	4-A-1 4-B-1 4-A-2
W1 3-18	Phenolic components of forages	Albany, Calif.	Yes	4-B-3
W1 3-19 (C)	Autoxidation of alfalfa lipids	Berkeley, Calif.	Yes	4-B-2
W1 3-20 (C)	Products from southeastern grasses	Tifton, Georgia	Yes	
W1 3-21 (C)	Alfalfa products	Lincoln, Nebr.	Yes	
W2 2-22 (Rev.)	Chemical treatment of wool for shrink resistance and other "easy-care" properties	Albany, Calif.	Yes	5-B-1 5-B-2 5-C-1 5-C-3 5-C-2
W2 2-24 (Rev.)	Effect of fabric construction and functional properties	Albany, Calif.	Yes	5-A-1
W2 2-28 (Rev.)	Mechanical behavior of wool fibers and fibrous assemblages	Albany, Calif.	Yes	

<sup>1</sup> Project discontinued during the reporting period.

<sup>2</sup> Recently initiated project.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress Area & Subheading	
W2 2-29 (Rev.)	Effects of radiation on natural and modified wools	Albany, Calif.	Yes	5-A-3
W2 2-32	New types of yarns and fabrics from coarse wools	Albany, Calif.	Yes	4-B-2
W2 2-35 (C) <sup>1</sup>	Wear-wrinkling performance of light weight wool fabrics	Washington, D.C.	Yes	5-C-2
W2 2-36 <sup>1</sup>	WURLAN treatment of wool top	Albany, Calif.	Yes	5-C-1
W2 2-37 (C)	High luster wool fabrics	Washington, D.C.	Yes	5-B-3
W2 2-38 (C)	High energy radiation of wool	Durham, North Carolina	Yes	5-B-4
W2 2-39	Functional blends of wool with other fibers	Albany, Calif.	Yes	5-B-1
				5-C-3
W2 2-40	Physical and chemical structure of wool	Albany, Calif.	Yes	5-A-1
				5-A-2
W3 1-112 (Rev.) <sup>1</sup>	Dried fruit products and processes	Albany, Calif.	Yes	7-C-1
W3 1-117 (Rev.)	Fruit pigments	Albany, Calif.	Yes	7-E-2
W3 1-121	Heat transfer surface fouling	Albany, Calif.	Yes	6-B-1
W3 1-123 (C)	Fruit leucoanthocyanins	Los Angeles, Calif.	Yes	7-C-1
W3 1-124 (Gr.) <sup>1</sup>	Cell wall organization of fruits	Cambridge, Mass.	Yes	7-C-2
W3 1-125	Composition of desert grapefruit	Pasadena, Calif.	Yes	6-A-2
				6-B-1
W3 1-126	Viniferous grape products	Albany, Calif.	Yes	7-B-2
				7-D-1
W3 1-127	New fruit dehydration methods	Albany, Calif.	Yes	7-B-2
				7-E-1
W3 1-128	Rancidity control in walnuts	Pasadena, Calif.	Yes	7-A-1
W3 1-129 (C)	Grape juice extraction	Davis, Calif.	Yes	7-E-3
W3 1-130	Date composition and products	Pasadena, Calif.	Yes	6-B-2
W3 1-131	Tropical fruit products	Honolulu, Hawaii	Yes	6-D-2
W3 1-132	Processing quality--Northwest fruits	Puyallup, Wash.	Yes	7-E-4
W3 1-133 (C)	Products from desert grapefruit	Tucson, Ariz.	Yes	6-D-1
W3 1-134 (C)	Phenolics in canned apple juice	Fort Collins, Colorado	Yes	7-C-1
W3 1-135	Grape product developments	Albany, Calif.	Yes	7-D-1
				7-E-3
W3 1-139	Composition of lemons and lemon products	Pasadena, Calif.	Yes	6-A-1
				6-D-1
W3 1-140 (C)	Improvement of canned ripe olives	Berkeley, Calif.	Yes	7-B-2
W3 1-141 (C)	Determination of bitter components in navel orange products	So. Pasadena, Calif.	Yes	6-A-2
W3 1-142 (C)	Improved fining methods for wine	Geneva, N. Y.	Yes	7-E-3
W3 1-143 (C)	Fluidized bed drying	Chicago, Ill.	No <sup>2</sup>	
W3 1-144	Navel orange bitterness	Pasadena, Calif.	Yes	6-A-2
				6-C-1
W3 1-145 (C)	Mechanically harvested dates	Riverside, Calif.	No <sup>2</sup>	
W3 1-146	Flavor of fruit products	Albany, Calif.	Yes	6-A-1
				7-B-1

<sup>1</sup> Project discontinued during the reporting period.

<sup>2</sup> Recently initiated project.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj., Incl. in	
			Summary of Progress	Area & Subheading
W3 4-79 (Rev.) <sup>1</sup>	Effects of processing on potato product flavor	Albany, Calif.	Yes	8-B-1 8-C-1 8-D-1 8-D-2 9-C-1
W3 4-80	Effects of processing operations upon texture of frozen vegetables	Albany, Calif.	Yes	9-C-1
W3 4-81 (C) <sup>1</sup>	Dry bean characteristics	Urbana, Ill.	Yes	9-A-1
W3 4-83	Mechanism of anhydration in bacterial spores	Albany, Calif.	Yes	9-D-1
W3 4-84 (C)	Histological studies of vegetables for dehydration	Davis, Calif.	Yes	9-C-2
W3 4-85 <sup>1</sup>	Flavor of tomato products	Albany, Calif.	Yes	9-B-1
W3 4-86	Chemistry and enzymology of vegetable flavors	Albany, Calif.	Yes	9-B-1 9-B-3 9-E-5
W3 4-87 (C)	Removal of radioactive fallout	Berkeley, Calif.	Yes	9-E-5
W3 4-88	Processing quality--Northwest vegetables	Puyallup, Wash.	Yes	9-E-2
W3 4-89 (C)	Dry bean and pea powder	East Lansing, Michigan	Yes	9-E-4
W3 4-90 (Gr.)	Sporulation of food spoilage bacteria	Urbana, Ill.	Yes	9-D-1
W3 4-91	Effects of processing variables on dehydrated vegetables	Albany, Calif.	Yes	9-C-3 9-E-1 9-E-3 8-A-1
W3 4-93 (Gr.)	Lipids in plant tissue	Davis, Calif.	Yes	8-A-1
W3 4-94	Control of microbial contamination	Puyallup, Wash. Albany, Calif.	Yes	7-D-1 9-D-1
W3 4-95	Potato products	Albany, Calif.	Yes	8-B-1 8-D-1 8-D-2 9-A-1 9-E-4
W3 4-96	Dry bean products and components	Albany and Pasadena, Calif.	Yes	9-A-1 9-E-4
W4 3-1 (Rev.)	Chemical derivatives of ricinoleic acid	Albany, Calif.	Yes	12-A-1 12-B-1 12-C-1
W4 3-2 (Rev.)	Foamed polyurethanes from castor oil	Albany, Calif.	Yes	12-C-1
W4 3-8	Castor pomace deallergenation	Albany, Calif.	Yes	11-A-1
W4 3-9 <sup>1</sup>	Safflower oil and meal	Albany, Calif.	Yes	11-A-1
W4 3-10 (C)	Antioxidants of safflower oil	Tucson, Ariz.	Yes	10-A-1
W4 3-11 (C)	Polymerization of vegetable oil-derived monomers	Tucson, Ariz.	Yes	12-B-1
W4 3-12 (C)	Continuous production of urethane foams	Minneapolis, Minnesota	Yes	12-C-1
W4 3-13	Stability of safflower oil	Albany, Calif.	Yes	10-B-1
W5 1-73 <sup>1</sup>	Biochemical studies of non-sucrose carbohydrates in sugar beets	Albany, Calif.	No <sup>3</sup>	
W5 1-75 <sup>1</sup>	Effects of non-sugar chemicals on processing	Albany, Calif.	No <sup>3</sup>	
W5 5-37 (Rev.) <sup>1</sup>	Evaluation of hydroxy-conjugated dienoic acid oils	Albany, Calif.	Yes	12-A-1 12-B-1 12-C-1
W5 5-46 (C) <sup>1</sup>	Preparation and evaluation of surface coatings	Fargo, North Dakota	Yes	12-C-1
W6 1-48 (Rev.) <sup>1</sup>	Chemistry of poultry flavor	Albany, Calif.	Yes	13-B-1
W6 1-59 (C)	Reduction of Salmonella contamination in egg products	Ames, Iowa	Yes	14-B-1

<sup>1</sup> Project discontinued during the reporting period.

<sup>3</sup> Utilization research on sugar beets has been discontinued.



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			Summary of Progress	Area & Subheading
W6 1-60 (C)	Histological study of frozen poultry	Madison, Wisc.	Yes	13-A-1
W6 1-61	Elimination of Salmonella in egg products	Albany, Calif.	Yes	14-C-1
W6 1-62	Freeze-dried poultry meat	Albany, Calif.	Yes	13-D-1
W6 1-64 (Gr.)	Salmonella metabolism	Ithaca, New York	Yes	14-B-1
W6 1-65 (C)	Freeze drying of poultry meat	Berkeley, Calif.	Yes	13-D-1
W6 1-66 (C)	Pasteurization of eggs	Davis, Calif.	Yes	14-C-1
W6 1-68	Egg proteins	Albany, Calif.	Yes	14-A-1
W6 1-69 (C)	Chemical, physical or enzymic modification of egg white	Ames, Iowa	Yes	14-C-1
W6 1-70	Heat resistance of Salmonella	Albany, Calif.	Yes	14-B-1
W6 1-71 (C)	Microstructure of poultry meat	Madison, Wisc.	No <sup>2</sup>	
W6 1-73	Poultry muscle proteins	Albany, Calif.	Yes	13-C-1 13-D-1
WU-P-1	Plant enzymes	Albany, Calif.	Yes	6-A-5
WU-O-0-2(OCD)	Fallout shelter foods	Albany, Calif.	Yes	1-B-2 1-E-1
WU-O-0-3(BA)	Flavor components of hop oil	Albany, Calif.	Yes	8-B-2
UR-A7-(10)-97	Toxicity of fungal metabolites	Madras, India	No <sup>2</sup>	
UR-A10-(10)-22	Rheology of wheat flour doughs	Haifa, Israel	Yes	1-C-3
UR-A11-(10)-20	Dough properties	Osaka, Japan	No <sup>2</sup>	
UR-E4-(10)-4	Soluble proteins in wheat	Antwerp, Belgium	No <sup>2</sup>	
UR-E9-(10)-2	Composition of whole wheat lipids	Paris, France	Yes	1-A-2
UR-E9-(10)-7	Immunochemical analysis of wheat and barley proteins	Paris, France	Yes	1-A-1
UR-E9-(10)-8	Solubility of wheat gluten proteins	Montpellier, France	Yes	1-C-2
UR-E9-(10)-43 <sup>1</sup>	Phosphorus in wheat flour	Paris, France	Yes	1-A-2
UR-E9-(10)-44	Ultrasonic study of wheat gluten	Paris, France	Yes	1-A-1
UR-E9-(10)-45	Enzyme action in low-moisture grain	Paris, France	Yes	1-A-2
UR-E15-(10)-31	Wheat germ proteins	Bologna, Italy	Yes	1-A-1
UR-E21-(10)-1 <sup>1</sup>	Sulfhydryl groups in wheat	Poznan, Poland	Yes	1-A-1
UR-E21-(10)-18	Coenzyme role of riboflavin of wheat endosperm	Poznan, Poland	Yes	1-A-2
UR-E27-(10)-1	Pentosans of wheat	Zurich, Switzerland	Yes	1-A-1
UR-E29-(10)-38	Separation of the total protein of wheat flour	St. Albans, England	Yes	1-A-1
UR-E29-(10)-47 <sup>1</sup>	Biological value of processed wheat	Cambridge, England	Yes	2-A-2
UR-E29-(10)-76	Bread dough interactions	Chorleywood, England	Yes	1-C-2

<sup>1</sup> Project discontinued during the reporting period.

<sup>2</sup> Recently initiated project.

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			Summary of Progress	Area & Subheading
UR-01- (10)-1	Dough rheology	North Ryde, Australia	Yes	1-C-3
UR-E15- (10)-17	Natural antioxidants in alfalfa	Milano, Italy	Yes	4-A-2
UR-E29- (10)-52	Structure of alfalfa polysaccharides	Edinburgh, Scotland	Yes	4-A-3
UR-A7- (20)-15	Molecular processes in wool	Allahabad, India	Yes	5-A-1
UR-A7- (20)-58	Adsorption of selected ions to wool	Ahmedabad, India	Yes	5-B-4
UR-E8- (20)-10 <sup>1</sup>	Finishing treatments for improved qualities in wool fabrics	Helsinki, Finland	Yes	5-B-4
UR-E10- (20)-8	X-ray diffraction patterns of wool	Aachen, West Germany	Yes	5-A-1
UR-E26- (20)-7	Sulfur in wool keratins	Stockholm, Sweden	Yes	5-A-1
UR-E29- (20)-56 (Rev.)	Chemical structure of wool protein	Leeds, England	Yes	5-A-2
UR-A6- (30)-3	Polysaccharides in plant cell walls	Taipei, Taiwan	Yes	7-C-2
UR-A7- (30)-56	Organic acids in fruits	Baroda, India	No <sup>2</sup>	
UR-A7- (30)-60	Fruit leucoanthocyanins	Delhi, India	Yes	7-C-1
UR-A7- (30)-64	Tannin degradation in fruits	Madras, India	No <sup>2</sup>	
UR-A10- (30)-32	Enzymatic browning in deciduous fruits	Jerusalem, Israel	Yes	7-C-1
UR-S5- (30)-2	Tropical fruit flavors	Bogota, Colombia	Yes	6-A-1
UR-A7- (30)-39	Bean proteins	Allahabad, India	Yes	9-A-1
UR-E8- (10,30)-15	Composition of vegetables and fodder	Helsinki, Finland	Yes	9-B-1
UR-E9- (30)-54	Enzymatic activities of bacterial spores	Paris, France	Yes	9-D-1
UR-E26- (30)-5	Autoxidation of fats in dehydrated vegetables	Gothenburg, Sweden	Yes	8-A-1
UR-E26- (30)-11	Role of metals in vegetable enzyme action	Gothenburg, Sweden	Yes	8-A-1
UR-A7- (40)-21	Hydroxylated derivatives of linseed and safflower oils	Hyderabad, India	Yes	12-B-1
UR-A7- (40)-69	Polymerizable monomers from castor oil	Hyderabad, India	Yes	12-B-1
UR-A7- (40)-107	Conversion of castor oil monoglycerides	New Delhi, India	No <sup>2</sup>	
UR-A7- (50)-31	Reaction of sucrose with sulfonyl chloride and other chemicals	Calcutta, India	No <sup>3</sup>	
UR-A10- (50)-25	Enzymatic sucrose degradation in sugar beet tissues	Jerusalem, Israel	No <sup>3</sup>	
UR-A7- (60)-27	Physicochemical properties of hen egg yolk proteins caused by freezing	Bangalore, India	Yes	14-C-1
UR-E9- (60)-76	Chemistry of egg lysozyme	Paris, France	Yes	14-A-1
UR-01- (60)-4	Ovalbumin in eggs	Ryde, New South Wales, Australia	Yes	14-A-1

<sup>1</sup> Project discontinued during the reporting period.

<sup>2</sup> Recently initiated project.

<sup>3</sup> Utilization research on sugar beets has been discontinued.







